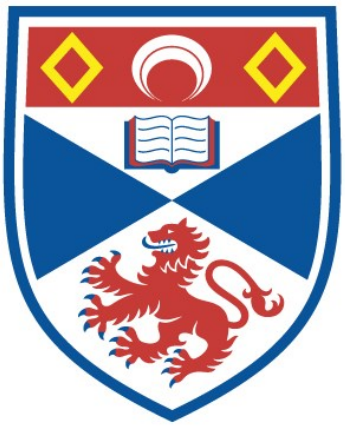
SCHOOLING DECISIONS AND DISCRIMINATORY ABILITIES OF FISH

Siân Wyn Griffiths

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Thesis submitted for the degree of Doctor of Philosophy,
University of St. Andrews

September 1997

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Hoffwn gyflwyno'r gwaith hyn i Wyn

COFIO

"Un funud fach cyn elo'r haul o'r wybren,
Un funud fwyn cyn delo'r hwyr i'w hynt,
I gofio am y pethau anghofiedig
Ar goll yn awr yn llwch yr amser gynt.

Fel ewyn ton a dyr ar drathell unig,
Fel cân y gwynt lle nid oes glust a glyw,
Mi wn eu bod yn galw'n ofer arnom -
Hen bethau angofiedig dynol ryw."

Waldo Williams

LIGHT IS LIKE WATER

"On Wednesday night, as they did every Wednesday, the parents went to the movies. The boys, lords and masters of the house, closed the doors and windows and broke the glowing bulb in one of the living room lamps. A jet of golden light as cool as water began to pour out of the broken bulb, and they let it run to a depth of almost three feet. Then they turned off the electricity, took out the rowboat, and navigated at will among the islands of the house."

Gabriel Garcia Márquez

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Abstract

The aim of this thesis has been to investigate the individual decision making abilities of schooling fish in order to understand the composition of fish schools. Do individuals choose particular school-mates, and if so, on what basis are these decisions made? The null hypothesis, that schools are composed of random assortments of individual fish, has been rejected. School membership and structure are profoundly affected by the cognitive abilities and partner choice decisions of fish.

Field work carried out in Trinidad and Dorset, UK (on guppies, *Poecilia reticulata*, and European minnows, *Phoxinus phoxinus*, respectively) has demonstrated that familiarity does indeed influence choice of schooling partner. Individual guppies under laboratory conditions and in the wild recognise and prefer school-mates with whom they are familiar, and schools of minnows are composed of a significant proportion of individuals which are familiar to one another. Preference for familiar conspecifics develops gradually (over at least 12 days in the case of guppies) and observations of wild guppies shows that this schooling preference is mediated by group size. Tendency to school with familiar fish is strong when group size is small, but declines thereafter, no preference being made as group size increases beyond ~40 individual females. The effect of group size on these partner choice decisions suggests that individual recognition may be possible. Intriguingly, a gender difference in the partner choice decisions of guppy schools in the wild has also been identified. Females spend more time schooling with individuals from their natural schools than males. This has important evolutionary consequences in terms of population differentiation and speciation. It seems, therefore, that schools are by no means composed of a random assortment of individuals. Indeed school structure and membership are profoundly affected by the remarkable discriminatory abilities of individual fish.

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CHAPTER 1

The adaptive value of schooling behaviour

1.1 Introduction: Why do fish school?

This thesis deals with the question of why fish swim together and in particular the cognitive ability which allows them to discriminate between potential schooling partners. Interest in why animals form groups has been foremost in the minds of behaviourists for many years and there are now known to be many advantages of living in groups (Hamilton 1971; Wittenberger 1981; Krebs & Davies 1987). Nevertheless, not all animals are gregarious and the propensity of some animals to aggregate more than others is one behavioural trait which has fuelled an extensive debate as to the level at which natural selection acts, not least of all in the field of fish schooling behaviour. For example, the release of Schreckstoff, a putative alarm substance, from the damaged skin of ostariophysan fish was cited as an example of altruism on the part of injured fish (von Frisch 1938; von Frisch 1941; Pfeiffer 1960). Its hypothesised function was to warn others in the group of a nearby predation risk (see Irving 1996; Magurran *et al.* 1996, for alternatives to this function in the wild). In this way, schools of fish were interpreted to be groups of individuals co-operating fully with one another for the good of the group (Breder 1954; Wynne-Edwards 1961; Shaw 1962; Radakov 1973).

The problem with accepting that natural selection acts at the level of the group is that the expression of any selfish behaviour by an individual will endow it with a competitive advantage over others, which in turn would soon be out-competed. Selection must act at the level of the individual, and the seemingly altruistic behaviour of animals towards one another - for example the alarm calls of Belding's ground squirrels, *Spermophilus beldingi*, (Sherman 1977; Sherman 1980) and Kloss's gibbons, *Hylobates klossii*, (Tenaza & Tilson 1977), the nest helping of white-fronted bee-eaters, *Merops bullockoides*, (Emlen & Wrege 1988) or blood sharing of female and male vampire bats, *Desmodus rotundus*, (Wilkinson 1984; DeNault & McFarlane 1995) may then be explained either by the theory of

kin selection (Haldane 1955; Hamilton 1964) or by the process of reciprocal altruism (Trivers 1971).

The theory of kin selection describes how the requirement for individuals to behave selfishly, if they are to avoid being out-competed, can be reconciled with observations of altruistic behaviour. By helping a relative, an individual is able to increase the chances that copies of a proportion of its own genes (as well as its relatives' genes) will be propagated (Haldane 1955; Hamilton 1964). In the case of fish, the most important direct benefits to an individual joining a school are anti-predator (Neill & Cullen 1974; Magurran 1990a; Pitcher & Parrish 1993) and foraging advantages (section 1.2, Pitcher *et al.* 1982; Clark & Mangel 1984; Krause 1993b). However, it is now known that, under certain circumstances, fish can distinguish kin from unrelated individuals (Quinn & Busack 1985; Brown & Brown 1992; Warburton & Lees 1996). For example, anadromous species are especially well known for the remarkable homing migrations which they are able to achieve by detecting population specific water-borne odourants (Hara 1993). More recent work has quantified the preference of salmonids such as coho salmon, *Oncorhynchus kisutch*, for related individuals under laboratory conditions (Courtenay *et al.* 1997). Are school members related to one another? If so then kin selection may be one explanation for the evolution of schooling behaviour. This is because individual fish are able to increase their inclusive fitness by behaving altruistically toward related conspecifics. Furthermore, the association of relatives within schools could effect a reduction in gene flow through the population which in turn would have important evolutionary implications.

Recent work on the cognitive abilities of animals has revealed a subtle way in which individuals may behave altruistically or co-operatively without the need to invoke the theory of kin selection. An increasing amount of evidence exists to demonstrate that individuals recognise particular conspecifics and remember their identity from one encounter to the next. Preferential association with these so called familiar individuals may allow a strategy of reciprocal altruism to be forged (Trivers 1971). For example, Milinski (1990a; 1990b) and Dugatkin & Alfieri (1991b) working on threespined sticklebacks, *Gasterosteus aculeatus*, and guppies, *Poecilia reticulata*, respectively, showed that these fish choose to

associate with the most co-operative of the individuals with whom they have inspected predators during previous encounters. The slow, saltatory movement of a fish away from a school and towards a predator during inspection behaviour is potentially fatal (Pitcher *et al.* 1986a; Dugatkin 1992) but see Milinski & Boltshauser (1995) and Godin & Davis (1995). However, the apparently altruistic act of adopting the lead position during inspections may be reciprocated in the future (see Dugatkin 1997, for a review of the prisoner's dilemma and tit-for-tat strategy). By choosing the same inspection partner on successive occasions the opportunity arises for each fish to alternately adopt the lead position. The preferential association of particular individuals in this way makes it possible for fish to gain schooling benefits additional to those associated with joining a randomly assorted group of conspecifics.

It seems, therefore, that fish have surprising cognitive abilities which play an important role in individual decision making. To what extent are fish able to implement these abilities? More importantly perhaps, what relevance do these decisions have for schooling dynamics in the wild? The aim of this project is to investigate the schooling decisions of individual fish both under laboratory conditions and in the wild. The null hypothesis is that fish schools are composed of random assemblages of individuals. If this is the case then individual fish would be predicted to choose school-mates at random and, all else being equal, movement between schools would also be independent of school membership. Schooling behaviour is a widespread phenomenon, with as many as 25% of 20,000 species of teleosts and 80% of total number of fish species schooling at some stage in their life cycle (Burgess & Shaw 1979). The implications of this work are therefore likely to be far reaching. Before outlining the steps taken during this project to investigate the question of whether schools are random assemblages of fish (1.4) an overview of the adaptive value of schooling will first be presented (1.2). Second, the additional benefits which might be gained by fish which choose to school with particular types of conspecific will be discussed (1.3).

1.2 Balancing the costs and benefits of schooling

There is a vast literature describing the function of schooling. The greater part of this has concentrated on schooling behaviour as an effective anti-predator and foraging strategy. However, moment to moment decisions as to whether to stay or leave a school are made according to the balance of costs and benefits to each fish of many influences which may also include parasite transmission and hydrodynamic efficiency. Reviews of the short term costs and benefits to individual fish of joining schools are given by Cushing & Harden Jones (1968); Pitcher *et al.* (1979); Partridge (1982); Godin (1986); Magurran (1990a) and Pitcher & Parish (1993). The longer term, evolutionary, implications of schooling behaviour are also briefly discussed below. Throughout this thesis differentiation between schooling (a synchronised and polarised group, Pitcher 1983) and the more broadly defined shoaling (Pitcher & Parrish 1993) is not made unless specifically qualified.

(a) Schooling as a defence against predation and parasitism

What role does schooling play in the interaction between piscivores and their prey? Schooling behaviour is a highly effective strategy against predators (reviewed by Godin 1986; Magurran 1990a; Pitcher 1992) despite the increased conspicuousness which a group may have compared to a solitary fish (Krause & Godin 1995). Indeed, the beautiful and highly co-ordinated manoeuvres of schools, which are especially well known in marine species including mackerel, *Scomber scomber*, and herring, *Clupea harengus* (Pitcher *et al.* 1985), are often implemented as counter predation measures (Pitcher & Parrish 1993).

One of the many anti-predator advantages individual fish derive from school membership includes increased vigilance (Magurran *et al.* 1985). The early detection of an approaching aquatic or aerial predator by a school of fish can be attributed to the increased vigilance of 'many eyes' (Bertram 1978; Magurran *et al.* 1985). For example, Godin *et al.* (1988) quantified the relationship between school size and level of group vigilance for the glowlight tetra, *Hemigrammus erythrozonus*. As group size increased, detection of a randomly allocated flash of light around the periphery of the tank increased. The same principle also applies to other group living animals including ostriches, *Struthio camelus*, (Bertram 1980)

and Thompson's gazelle, *Gazella thomsoni*, (Fitzgibbon 1989) as modelled by Lima (1989)

The increased vigilance of larger schools allows fish to delay their flight response and continue activities such as foraging because the predator is more likely to have been detected at an early stage in its attack sequence (Pitcher 1986; Ydenberg & Dill 1986). However, another possible advantage lies in the increased time available for assessment of the predator and the nature of the threat it poses. This assessment can be achieved by predator inspection behaviour and is undertaken most often by fishes subject to stalking or ambush predation. For example, European minnows, *Phoxinus phoxinus*, in small groups or as individuals leave the main school, approach the predator to within 4-6 body lengths before pausing and then swimming to rejoin the remaining school (Magurran & Pitcher 1987). Although one of the initial functions of inspection behaviour is predator recognition, the motivational state of the predator may also be revealed (Magurran 1986b). If this is the case, the cost of inappropriate false alarms and flee responses will be avoided. The trade-off between obtaining information and risk of being eaten when approaching a potential predator will obviously vary with inspection group size, because an individual inspector will be more at risk from attack than two or more inspectors.

In addition to the benefits of increased vigilance gained by schooling fish, the dilution (Bertram 1978; Mace 1983), selfish herd (Hamilton 1971) and confusion effects (Major 1978; Ohguchi 1981) may combine with group anti-predator manoeuvres (such as flash expansion, Magurran & Pitcher 1987), to diminish the risk to individual school members once an attack has been launched. If a predator is only able to kill one prey animal per successful attack, then the anti-predator advantages of schooling increase with group size due to the dilution effect. The chances that any one individual will be killed during an attack decreases with increasing group size because there is a good chance that another individual will be killed (Bertram 1978). For example, Foster & Treherne (1981) demonstrated that the attack rate of a predatory sardine fish, *Sardinops sagax*, on groups of water striders, *Halobates robustus*, was about the same for different sized groups. Because the fish predator attacks the insects from below (so that water strider vigilance is not influenced by

group size) it can be shown that the attack rate per individual only varies because of the dilution effect. However, this assumes that position within a group has no effect on strike probability. In fact, centrally located individuals might be at lower risk of predation than those at the periphery of the group. Individuals in the so called 'selfish herd' seek cover behind other individuals by trying to obtain positions in the centre of the group (Hamilton 1971). Selfish behaviour is thus one mechanism through which group formation is selected.

One of the first studies to suggest that schooling minimises the risk of death due to predatory attack in the wild was undertaken by Seghers (1974b) on the Trinidadian guppy. Six streams in Trinidad were ranked according to their predator abundance, and the cohesion of the guppy schools in each stream was measured. A positive relationship between school cohesion and predator abundance was found. Since then, the attack success of many aquatic predators including squid, *Loligo vulgaris*, cuttlefish, *Sepia officinalis*, pike, *Esox lucius*, and perch, *Perca fluviatilis*, (Neill & Cullen 1974), sticklebacks (Milinski 1979) and piranhas, *Serrasalmus* spp. (Tremblay & FitzGerald 1979) has been shown to decline with increasing group size. Furthermore, fish which become separated from the group are more likely to be eaten. This has been demonstrated in minnows (Magurran & Pitcher 1987), guppies (Godin & Smith 1988) and silversides, *Menidia menidia*, (Parrish 1989a).

The risk to an individual of parasite attack (although not parasite transmission) is reduced by the dilution and selfish herd effect in the same way as risk of predator attack (Pulliam & Caraco 1984; Godin 1986). However, whereas one predation attack may have fatal consequences, parasites may attack an individual many times without effecting its death (Giles 1983). Schooling behaviour decreases the number of parasites per school member (Poulin & FitzGerald 1987; Poulin 1991). Furthermore, individual fish can recognise and preferentially avoid parasitised conspecifics (threespine sticklebacks, Dugatkin *et al.* 1994). Central individuals are less likely to be parasitised than those at the periphery (Newson *et al.* 1973; Krause & Godin 1994a) which must trade the costs of a high parasite load in order to obtain the other benefits of schooling (Krause 1993c; Krause 1994; Krause & Tegeder 1994). If these other benefits are small, for example if anti-parasite tactics are the primary reasons for

schooling, then the costs of bearing a parasite load will not exceed that expected for a solitary individual without the group dispersing. Conversely, any anti-parasite benefits of schooling in a central position are traded against the costs to each individual of parasite transmission, as parasites and disease transmitted by contact or close proximity will be distributed more quickly through groups (a review of these costs and benefits is given by Magurran 1993; Krause & Godin 1994b).

(b) The foraging costs and benefits of schooling

Under the conditions peculiar to aquatic environments visibility may be restricted and food availability may be patchy. It then becomes advantageous for individual fish to become members of a school since location of food patches is achieved faster with increasing school size, as has been observed in minnows, goldfish, *Carassius auratus*, (Pitcher *et al.* 1982) and stone loach, *Noemacheilus barbatulus*, (Street & Hart 1985). The ability of an individual to monitor cues from the other school members is of great importance in order that it may join the food finder and gain as large a share of the available food as possible. The cues used by fish vary between species but include the 'nose down' feeding posture of goldfish and the 'wriggling' of minnows (Magurran & Pitcher 1983). Information gathered by monitoring these cues is transferred more quickly in larger schools. Pitcher & Magurran (1983) undertook reciprocal transfers of single 'informed' goldfish (which had knowledge of the location of good food patches) between schools foraging over different food patch arrangements. Goldfish in schools of five spent a greater proportion of their time foraging, undertook more sampling and were better at adjusting their foraging to changing patch profitability than schools of two (Pitcher & Magurran 1983).

Not only are fish able to find food faster when they become members of a school, but because of the anti-predator advantages of schooling, individuals are less wary (Seghers 1981) and may therefore spend a larger proportion of their time foraging (Bertram 1978). Magurran & Pitcher (1983) investigated foraging behaviour of individual goldfish and European minnows in single species schools of 2, 4, 6, 12, & 20 fish. They showed that timidity increased with decreasing school size, and that lengthy visits to the food patch were only made by individuals in larger schools. Thus fish in small schools will be at a disadvantage especially

when food is scarce because time allocated to foraging is lower than for larger schools and because time taken for discovery of the food will be greater than for a large school (Pitcher *et al.* 1982).

The foraging benefits afforded to school members are not distributed equally between individuals. Indeed, fish adopt positions within schools that provide most foraging advantages if other factors are equal (Krause *et al.* 1992). Krause *et al.* (1992) demonstrated that juvenile roach, *Rutilus rutilus*, have positional preferences within a school which lead to hungry individuals occupying front positions where they gain foraging advantages.

While the benefits to an individual of joining a school may increase with increasing school size, the foraging advantage begins to decrease when school size increases beyond a critical level. Competition for food, and therefore costs of group living increases with increasing benefits (Bertram 1978). Goldfish in schools of 20 spend no more time foraging than goldfish in schools of 12 (Magurran & Pitcher 1983), and Street *et al.* (1984) found that this increase in competition was reflected by a decrease in handling time per food item, a behaviour that has metabolic costs for the individual.

(c) Hydrodynamic benefits of schooling

The classic view of energy expenditure of fish living in schools suggests that individuals in schools have a reduced energetic cost of locomotion compared to solitary individuals (Belyayev & Zuyev 1969; Weihs 1973; 1975). However, experimental evidence on schools of saithe, *Pollachius virens*, herring and cod, *Gadus morhua*, (Partridge & Pitcher 1979; Partridge *et al.* 1983) does not provide evidence of the specific three-dimensional school structure that these theoretical models predict. Nevertheless, recent work by Abrahams & Colgan (1985; 1987) observed that shiners, *Notropis heterodon*, formed 2-dimensional schools in the absence of predators, and in doing so became more hydrodynamically efficient. In environments where predators were present, however, 3-dimensional schools were formed in order that vigilance could be maximised. It remains to be seen, then, whether improved theoretical models of school structure will more closely match observed patterns of schooling behaviour.

(d) The evolution of schooling behaviour

How do the short term costs and benefits of group living translate into evolutionary change? Haskins & Haskins (1951) worked on guppy populations in Trinidad and the guppy has become a classic example of evolution in action. It is known that genetic variation within a population allows natural selection to operate. The Haskinses realised that morphological variation in male colour patterns was large between populations. Males from sites where predator pressure was strong were not as colourful as males observed in habitats (usually upstream) where predators were rarely found. Haskins & Haskins (1951) transplanted 200 adults in 1957 from a region of the Caroni drainage (where predation pressure is relatively high) into a guppy and predator free Oropuche drainage (described in chapter 2). Since then, colonisation has been achieved at the original position of release and also downstream (where predators are present). Magurran *et al.* (1992) investigated the behavioural changes in the transplanted guppies resulting from this alteration in predation pressure. It was found that schooling tendency decreased at the site of first release because predation pressure had also decreased, but that at the downstream site schooling tendency had increased once more (Magurran *et al.* 1992). Thus an evolutionary response to short term changes in costs and benefits of schooling has been documented to occur within 100 generations. Furthermore it was shown that anti-predator behaviour was an inherited behavioural pattern and that it had been modified over time by selection (Magurran *et al.* 1992).

Another example of natural selection acting on variance in genotypes so as to affect behavioural changes is given by Magurran & Seghers (1990a). They demonstrated that population differences in predator recognition in the guppy (measured as schooling tendency) could be attributed to the different types of predator to which each population was accustomed in the wild (Magurran & Seghers 1990a). For example at the lower Aripo River a range of piscivores exist (including charachids and cichlids) whereas the Paria River contains only *Macrobrachium* prawns and is therefore a low predation pressure site. Schooling is well developed in guppy populations living in rivers with a high density of predators but only poorly developed in populations living in rivers with only low densities of predators. Similarly, Magurran & Pitcher (1987) compared the anti-predator tactics (repertoire & effectiveness) of minnow schools

obtained from different populations in the UK. Minnows sympatric with an ambush predator (the pike) were compared to minnows which do not co-occur with piscivores. It was found that those fish sympatric with pike were able to integrate their tactics more effectively (due to the selection pressure exerted upon them over many generations) than the minnows from other populations (Magurran & Pitcher 1987; Magurran 1990b). Fréon (1998) highlights another way in which behaviour is influenced by genetics, that is by comparing hybrids (Parzefall 1986). Two populations of fish differing in their schooling behaviour were investigated to see if these differences could be attributed to genetic change. Individual *Astynax mexicanus* from a cave population (which were blind and did not school) were crossed with conspecifics from populations where schooling tendency was much higher. The hybrids, which were not blind, nevertheless demonstrated decreased schooling tendency so that Parzefall (1986) could conclude that there was a genetic basis to the reduction in schooling behaviour for these wild cave dwelling populations of fish.

1.3 Do some fish make better schooling partners than others?

In recent years it has become evident that fish can discriminate amongst conspecifics and that this ability plays an important role in individual decision making (reviewed by Pitcher 1992; Dugatkin & Wilson 1993; Dugatkin & Sih 1995; Dugatkin 1997). Fish can easily distinguish amongst conspecifics on the basis of obvious morphological differences. For example, school-mates of a similar size are preferred by mackerel and herring (Pitcher *et al.* 1985), minnows (Pitcher *et al.* 1986b; Theodarkis 1989) and threespine sticklebacks (Ranta *et al.* 1992a; 1992b; Krause *et al.* 1996b). Similarly female guppies use colour cues to facilitate choice of mate (Houde 1987; Houde & Endler 1990). Some cyprinids also have good colour vision, which is often used in bright and conspicuous mating displays by, for example, redside shiners, *Richardsonius baleatus,* bluenose shiners, *Notropis welaka,* and rosyface shiners, *N. rubellus* (Smith 1991). Distinction between individuals on the basis of past experience in association with specific cues is known as condition dependent recognition (see section a, below). An overview of recent studies giving evidence for partner choice by fish in the contexts of anti-predator and foraging

behaviour, as well as in kin selection is presented by Dugatkin & Wilson (1993) and further examples from many different taxa are described by Dugatkin & Sih (1995). However, discrimination between conspecifics on the basis of previous experience only (condition independent recognition, see section b, below) is also possible. Individuals which are recognised on the basis of previous experience alone are usually termed familiar conspecifics.

(a) Condition dependent recognition

Fish are able to discriminate amongst particular individuals over a very short time frame. Milinski *et al.* (1990b) investigated the predator inspection behaviour of sticklebacks that had been kept in groups of four for a 'few' hours. The fish tended to perform predator inspection behaviour in pairs, and they found that in all cases at least some of the individual fish had partners with whom they preferentially inspected the predator. Individuals were found to prefer the same partner significantly more often than expected by chance. In a separate experiment, Milinski (1990a) showed that sticklebacks could remember the better of two inspectors after seeing each of them inspect just four times. Dugatkin & Alfieri (1991b) similarly found that guppies preferred to associate with the better of two inspectors. Individual guppies are able to identify and remember the more co-operative conspecific in a predator situation and preferentially associate with this individual in future encounters staged 4 hours later. Three fish were placed in parallel channels and had one minute in which to settle and a further 2.5 minutes in which to inspect a predator. Immediately afterwards the central fish was given a choice test in which it had the opportunity of associating with either of its former inspection partners. In 80% of trials there was a preference for the individual that had spent most time close to the predator. This level of discrimination persisted even if partner preference was tested 4 hours after the inspection test, rather than at once.

These tests reveal that individual fish can readily distinguish between conspecifics but do not prove that this discrimination is based on familiarity alone. Although Milinski and his colleagues (Milinski *et al.* 1990b) suggested that the quickly-established preference they saw was evidence for individual recognition, they could not exclude the possibility that the test sticklebacks had learnt to recognise the position of the better

inspector rather than its actual identity. It may be that the selection of partners during predator inspection is a form of condition-dependent recognition.

Support for the idea that fish assess their inspection partners on the basis of condition comes from an investigation by Külling & Milinski (1992). They showed that sticklebacks preferred to inspect in the company of larger individuals. Large individuals are preferred by the predator and are therefore more likely to distract the predator's attention from the smaller partner. Evidence clearly exists, therefore, to demonstrate an anti-predator advantage to schooling with particular conspecifics, and schools of familiar fish demonstrate better anti-predator behaviours (Chivers *et al.* 1995). Furthermore, foraging advantages are also available to fish that preferentially associate with poor competitors (Metcalfe & Thomson 1995).

Many fish are now understood to have the cognitive ability to distinguish kin from non-kin (table 1), although work to date has concentrated on salmonids. For example, Quinn & Busack (1985) demonstrated that juvenile coho salmon, *Oncorhynchus kisutch,* prefer water conditioned by both familiar and unfamiliar siblings over non-siblings. Similarly, Brown & Brown (1992) found that both juvenile Atlantic salmon, *Salmo salar,* and rainbow trout, *Oncorhynchus mykiss,* can discriminate kin from non-kin, preferring water conditioned by kin in a two-choice tank. Recently, work by Warburton & Lees (1996) has demonstrated that kin discrimination may also be possible for a domestic strain of guppy.

Although it is clear that fish are able to distinguish relatives from unrelated conspecifics and despite the apparent evolutionary advantages to an individual of choosing to school with kin (Blaustein *et al.* 1988), there is little evidence to date to support the idea that wild schools are composed of related individuals (Avise & Shapiro 1986; Naish *et al.* 1993). However, fish may vary greatly in their capacity to form schools of related individuals. Avise & Shapiro (1986) assessed kinship within groups of coral reef fish, *Anthias squampinnis,* whose larvae are dispersed during a pelagic phase. It is perhaps not surprising, then, that these individuals did not reunite as adults, and it is possible that fish species with different life

histories (e.g. livebearers or mouth brooders) may yet provide examples of relatedness within schools.

Table 1. Summary of investigations of the role of kinship in fish schooling decisions. Preferential association with kin was present (Y) or absent (N). The possible confounding effect of familiarity on these results are indicated as follows: test and stimulus fish housed together before trial and therefore possibly familiar with one another (F), naturally occurring groups of fish taken from the wild (W), test and stimulus fish were parent and offspring (P/O), levels of familiarity between stimulus fish controlled (C), and unknown (?).

Author(s)	Year	Common name	Scientific name	Preference for kin	Level of familiarity
Avise & Shapiro	1986	serranid reef fish	*Anthias squamipinnis*	N	W
Barnett	1982	midas cichlid	*Cichlasoma citrinellum*	Y	P/O
Brown & Brown	1992	rainbow trout	*Oncorhynchus mykiss*	Y	C
		Atlantic salmon	*Salmo salar*	Y	C
Brown & Brown	1993a	rainbow trout	*Oncorhynchus mykiss*	Y	F
		Atlantic salmon	*Salmo salar*	Y	F
Brown & Brown	1993b	rainbow trout	*Oncorhynchus mykiss*	Y	C
		Atlantic salmon	*Salmo salar*	Y	C
Brown *et al.*	1993	rainbow trout	*Oncorhynchus mykiss*	Y	C
Brown *et al.*	1996	Arctic charr	*Salvelinus alpinus*	Y	C
Courtenay *et al.*	1997	coho salmon	*Oncorhynchus kisutch*	Y	C + F
Dowling & Moore	1986	common shiner	*Notropis cornutus*	N	W
Ferguson & Noakes	1981	common shiner	*Notropis cornutus*	Y	W
FitzGerald & Morrissette	1992	threespine stickleback	*Gasterosteus aculeatus*	Y & N	C
Folke *et al.*	1992	Baltic salmon	*Salmo salar*	Y	F
Groot *et al.*	1986	sockeye salmon	*Oncorhynchus nerka*	Y	F
Loekle *et al.*	1982	guppy	*Poecilia reticulata*	Y	P/O
		black molly	*Poecilia sphenops*	Y	P/O

Author(s)	Year	Test species	Scientific name	Preference for kin	Level of familiarity
Magurran *et al.*	1995	Trinidadian guppy	*Poecilia reticulata*	Y	W
McKaye & Barlow	1976	midas cichlid	*Cichlasoma citrinellum*	Y	P/O
Moore *et al.*	1994	Atlantic salmon	*Salmo salar*	Y	F
Myrberg	1975	coral reef fish	*Cichlastoma nigrofasciatum*	Y	P/O
Naish *et al.*	1993	European minnow	*Phoxinus phoxinus*	N	W
Olsén	1986	Arctic charr	*Salvelinus alpinus*	Y	F
Olsén	1989	Arctic charr	*Salvelinus alpinus*	Y	C
Olsén *et al.*	1996	brown trout parr	*Salmo trutta*	Y	C
Quinn & Busack	1985	coho salmon	*Oncorhynchus kisutch*	Y	C
Quinn & Hara	1986	coho salmon	*Oncorhynchus kisutch*	Y	F
Quinn & Tolson	1986	coho salmon	*Oncorhynchus kisutch*	Y	C
Quinn *et al.*	1994	coho salmon	*Oncorhynchus kisutch*	Y	F
Stabell	1982	Atlantic salmon parr	*Salmo salar*	Y	F
Stabell	1987	Atlantic salmon parr	*Salmo salar*	Y	?
Van Havre & FitzGerald	1988	threespine stickleback	*Gasterosteus aculeatus*	Y	C
Winberg & Olsen	1992	Arctic charr	*Salvelinus alpinus*	Y	C
Warburton & Lees	1996	domestic guppy	*Poecilia reticulata*	Y	F

(b) Condition independent recognition

Further to the benefits that fish gain when they preferentially associate with the most co-operative of predator inspectors, or the poorest competitors, it is now clear that individual members of groups can enhance their fitness even further if they associate with familiar conspecifics with which they have associated on previous occasions. This effect was highlighted by Chivers *et al.* (1995) who discovered that schools of familiar fathead minnows, *Pimephales promelas*, were more cohesive and increased their co-operative anti-predator behaviour compared with schools comprised of unfamiliar individuals. Furthermore, European minnows prefer to associate with poor competitors since they may gain foraging advantages by doing so (Metcalfe & Thomson 1995). Previously, scant attention had been paid to how school membership influenced the effectiveness of schooling as an anti-predator or foraging device. The ability to discriminate familiar from unfamiliar conspecifics has now been demonstrated in a wide variety of fish species (table 2) including bluegill sunfish, *Lepomis macrochirus*, (Brown & Colgan 1986), threespine sticklebacks (Van Havre & FitzGerald 1988); Trinidadian guppies (Magurran *et al.* 1994) and fathead minnows (Brown & Smith 1994), implying that individual recognition is a neglected aspect of schooling dynamics.

Recognition of familiar conspecifics may be beneficial but how is it achieved? The oddity effect selects against individual variation in appearance because individual fish, which differ in appearance or behaviour, may be at greater risk of predation than their school-mates (Landeau & Terborgh 1986). Natural schools of fish are perhaps best known for the degree to which the school members resemble one another. Thus the very factor that confers protection, similarity in appearance, is also one that makes individual recognition more challenging.

It is hypothesised that recognition on the basis of familiarity, as opposed to condition-dependent recognition, takes a number of days to develop. Indeed, previous investigations of the role of familiarity in decision-making have examined groups of fish that were kept together for considerable periods of time. For example, Magurran *et al.* (1994) looked at schooling preferences for familiar fish in groups of less than 15 guppies that been together for two months. Brown & Smith (1994) and Chivers *et al.*

(1995) collected naturally occurring schools of fathead minnows from the wild and then maintained the schools in separate aquaria until they were ready to be tested. Metcalfe & Thomson (1995) examined the schooling preferences of European minnows kept in groups of seven for between 12 and 20 days (N. B. Metcalfe, pers. comm.). Dugatkin & Wilson (1992) found that bluegill sunfish that had been in groups of six for more than 3 months preferred to associate with familiar individuals.

If familiarity does take a number of days to develop then we might expect fish to associate with particular individuals for protracted periods in the wild. However, there have been few attempts to investigate the schooling preferences of wild fish and the results of these studies are equivocal. Helfman (1984) observed the behaviour of 102 individually identifiable yellow perch, *Perca flavescens* in a population in Cazenovia Lake, New York, which ranged from 107 to 445 fish. He found little tendency towards associations among particular individuals.

Table 2. Summary of investigations of the role of familiarity in fish schooling decisions. Evidence of discrimination between familiar and unfamiliar individuals was present (Y), absent (N) or unknown (?). The time for which test and stimulus fish were housed together (i.e. over which familiarity developed) is given in days or weeks. W indicates that test and stimulus fish were originally members of naturally occurring wild schools. The number of individuals housed together before trials were conducted is given by group size.

Author(s)	Year	Test species	Scientific name	Preference for familiar	Level of familiarity	Group size
Brown & Colgan	1986	bluegill sunfish	*Lepomis macrochirus*	Y	W + 3-7 days	5-8
		pumpkinseed sunfish	*Lepomis gibbosus*	N	W + 3-7 days	5-8
		rock bass	*Ambloplites rupestris*	N	W + 3-7 days	5-8
Brown *et al.*	1993	rainbow trout	*Oncorhynchus mykiss*	N	?	?
Brown & Smith	1994	fathead minnows	*Pimephales promales*	Y	W + 4 weeks	>25
Chivers *et al.*	1995	fathead minnows	*Pimephales promales*	Y	?	?
Dugatkin & Wilson	1992	bluegill sunfish	*Lepomis macrochirus*	Y	3 months	6
Griffiths & Magurran	1997a	Trinidadian guppy	*Poecilia reticulata*	Y	12 days	6
Griffiths & Magurran	1997b	Trinidadian guppy	*Poecilia reticulata*	Y	W	8-36
				N	W	62-194
Griffiths	1997	European minnow	*Phoxinus phoxinus*	Y	W + 3 days	70
Hilbourne	1991	skipjack tuna	*Katsuwonus pelamis*	N	W	
Magurran *et al.*	1994	Trinidadian guppy	*Poecilia reticulata*	Y	2 months	≤15
Metcalfe & Thomson	1995	European minnow	*Phoxinus phoxinus*	Y	12-20 days	7
Van Havre & FitzGerald	1988	threespine stickleback	*Gasterosteus aculeatus*	Y	W + 2 weeks	>100
Warburton & Lees	1996	domestic guppy	*Poecilia reticulata*	Y	1 week	8-10

1.4 The schooling decisions and discriminatory abilities of fish: the aims of this thesis

Although the field of cognitive ethology has been expanding rapidly, fish have been overlooked, and their cognitive abilities have been underestimated in the past. It is for this reason that this thesis will focus upon the individual decision-making abilities of schooling fish. Are individual fish able to discriminate between potential schooling partners, and upon which factors are these schooling decisions based? Trinidadian guppies and European minnows are used as model species to test the null hypothesis that schools are random assemblages of individuals. Chapter 2 gives a description of these study species and the field sites in which they were observed. A combination of laboratory studies (in the aquaria at the University of St Andrews) and field work (in Trinidad and in the River Frome at the Institute of Freshwater Ecology, Dorset, UK) has been used to ask if familiarity influences the choice of schooling partner (chapters 3 and 4). Familiarity is one way in which non-random associations between individuals may be formed. Familiarity is a prerequisite to co-operation and may therefore be important in the evolution of schooling behaviour. Until now most investigations of schooling have been conducted in confined laboratory settings. This project has been unique in also quantifying the behaviour of fish in the natural environment. Under such circumstances, fish may often encounter and be attacked by piscivorous predators. Chapter 4 investigates the question of whether preference for familiar conspecifics varies with risk of predation.

What other factors potentially influence the preference for familiar school members? Does gender also mediate schooling decisions? It is already known that schooling tendency may differ between the sexes, especially for species that are sexually dimorphic such as the guppy. This is partly since fecundity is related to longevity for female fish, so that anti-predator behaviour such as schooling is therefore of greater importance to females. Further anti-predator advantages are afforded to fish choosing to school with familiar individuals (Chivers *et al.* 1995). Because it is females which stand to benefit most from these additional advantages it may also be females which discriminate most strongly between familiar and unfamiliar school-mates. Chapter 5, therefore, tests the hypothesis that

sexual asymmetry in mating costs affects partner choice decisions during schooling in wild fish.

How long does it take for fish to be able to discriminate between conspecifics on the basis of familiarity alone? This question is important because although individual recognition may be beneficial, little is known of how it is achieved. Chapter 6 describes an experiment which measures the acquisition of familiarity by school members. One of the most striking features of natural schools is the degree to which school-mates resemble one another: a factor which, paradoxically, must make recognition difficult. If individual recognition plays a role in the discriminatory abilities of fish, then the ability of fish to distinguish between particular individuals may decline gradually with increasing group size. In chapter 7, therefore, the possibility that the number of potential schooling partners mediates schooling preferences in the wild is investigated. Is the expression of familiarity constrained by group size, or is recognition of conspecifics achieved by discrimination on the basis of a shared group characteristic?

Although non-random association of fish within schools may be due at least in part to the preference of fish for familiar conspecifics, the possibility that recognition is being achieved on the basis of kinship instead of, or as well as, familiarity cannot be discounted. Chapter 8 aims to investigate the degree to which relatedness and familiarity play a role in the partner choice decisions of fish. The guppy is a good species with which to test these possibilities as it is a livebearing fish where juveniles school from birth. The opportunity exists, therefore, for siblings to associate with one another and relatedness within schools may be relatively high. Furthermore, the experiment described in chapter 8 goes some way to elucidating the mechanism by which fish achieve discrimination among conspecifics. To what extent might chemosensory and visual cues provide information regarding school-mate identity? Lastly, chapter 9 considers the wider implications of the results of this thesis and discusses possibilities for future work.

CHAPTER 2

Study species and field sites

The experiments described in this thesis are all investigations of the schooling behaviour of either the European minnow, *Phoxinus phoxinus*, or the Trinidadian guppy, *Poecilia reticulata*. These two fish were chosen as suitable species for the study of schooling behaviour because they are found in large numbers in the wild and readily form discrete schools. Both species are also easy to keep and maintain under laboratory conditions in aquaria. Indeed, the guppy is extremely popular with aquarists who have succeeded in breeding fish with extraordinarily colourful fins. A large body of literature exists describing the biology of minnows and guppies, and therefore only a brief outline of the distribution and behaviour of each of these teleost fish will be provided here. This chapter will also give details of the field sites from which experimental animals were obtained and at which behavioural observations were made in the wild.

2.1 The European minnow

(a) Minnow life history and biology

The European minnow is a member of the family cyprinidae, which is the largest family of freshwater fish (Nelson 1994). Minnows therefore bear many features characteristic of cyprinids including slight lateral compression and presence of cycloid scales over the body (plate 1, Wheeler 1978; Allan 1986a; Winfield & Nelson 1991). Typically individual minnows reach a total length of no more than 7cm and rarely exceed a life span of 3 years (Pitcher 1971). The generalist feeding habit of the minnow is made evident by its terminal mouth and streamlined body shape. More direct evidence is presented by studies of gut content which show that the diet of minnows comprises a high proportion of algae and benthic invertebrates including, in the River Brathay, Cumbria, Ephemeroptera, Trichoptera, Chironomid larvae and filamentous algae (Frost 1943; Hartley 1948; Maitland & Campbell 1992).

Plate 1. European minnows in a laboratory aquarium

Males and females are similar in appearance except during the breeding season when the belly and fins of males become red, while females remain olive brown in colour. At this time (April to June) males may also develop white tubercles on the face and pectoral fins (Collette 1977). These increase the tactile effect of butting and mating embraces (described in fathead minnows, *Pimephales promelas*, by McMillan & Smith 1974) which occur during spawning (Smith 1991). Although individual males defend territories over areas of gravel, spawning is best described as communal (Maitland 1972; Winfield & Nelson 1991). Eggs are laid so that they adhere directly onto the gravel. Once hatched, the young are able to assume free swimming behaviour but schooling is not fully developed until approximately 4 weeks after this time (Magurran 1986a). It is thought that juvenile minnows (and many other 0+ cyprinid fishes) remain in marginal vegetation of shallow water (nursery areas) whilst juveniles (Garner 1996), only emerging into more open fast flowing water as they mature (Maitland 1972).

Minnows are a schooling fish which form single-species or mixed-species schools (Allan & Pitcher 1986). In a study of dace, *Leuciscus leuciscus*, gudgeon, *Gobio gobio*, and minnows in the River Frome, Dorset, schools were composed of all three fish, although individuals within each school tended to prefer conspecific neighbours (Allan 1986b). Because minnows are of no commercial importance in the U.K. and Europe, and because they can be found in large numbers and in discrete schools, they are a valuable species for the study of schooling behaviour in the wild. A large body of information describing cyprinid (including minnow) schools and schooling behaviour exists (e.g. Smith 1991). Many other aspects of minnow schooling behaviour including anti-predator behaviour (Magurran *et al.* 1985; Magurran 1986b; Magurran & Pitcher 1987; Magurran 1989b), foraging behaviour (Freeman & Grossman 1992), homing tendency (Kennedy & Pitcher 1975; Kennedy 1981), school structure (Pitcher 1973b; Pitcher 1973a; Naish *et al.* 1993) and response to the putative alarm pheromone, Schreckstoff, (Levesley & Magurran 1988; Magurran 1989a; Magurran *et al.* 1996; Irving & Magurran 1997) have also been investigated (see chapter 1).

(b) Minnow distribution and study site

The European minnow is common throughout rivers in Europe and Asia; from Ireland (where it was introduced, Maitland 1972) eastwards to the former Soviet Union, and from Finland and northern Sweden to the Pyrenees and Alps (Wheeler 1978; Winfield & Nelson 1991). In Britain minnows can be found in England, Ireland, Wales, and mainland Scotland (Maitland & Campbell 1992).

The River Frome in Dorset (NGR: SY 872870, figure 1) is an example of a habitat in which minnow behaviour and ecology has been especially well documented. The Institute of Freshwater Ecology (IFE) River laboratory has been situated on the banks of the River Frome at East Stoke near Wareham since 1963 (figures 1 & 2). As a result much chemical, physical and biological information pertaining to the Frome catchment has been collected. The River Frome is a typical lowland chalk stream (Westlake *et al.* 1972) which flows eastwards from springs in North Dorset near Evershot to the harbour at Poole. It flows for a distance of 65km through pastures and arable fields (Mann 1989), and supports the growth of a large amount of aquatic macrophytes, especially *Ranunculus*. Ibbotson *et al.* (1994) describe the river as moderately regulated. Some sections are heavily managed and channellised, and instream and bankside vegetation is cut regularly: partly so that cattle are able to reach the water's edge. Other parts of the river are not subject to these management measures, so that bankside and instream vegetation is allowed to grow freely (Ibbotson *et al.* 1994).

Minnows in the River Frome co-occur with other cyprinids including dace, gudgeon and roach, *Rutilus rutilus*. Other fish present include salmon, *Salmo salar;* brown trout, *Salmo trutta;* European eel, *Anguilla anguilla;* stone loach, *Noemacheilus barbatulus;* bullhead, *Cottus gobio* and pike, *Esox lucius*. Predators of River Frome minnows include pike, eels and also aquatic birds such as heron, *Ardea cinerea,* and kingfishers, *Alcedo atthis*. The pike is generally considered to be the most important and the most numerous piscivorous predator: reaching densities of $0.015m^{-2}$ (Mann 1980). Minnows comprise up to 50% of the diet of young pike in Southern England (Mann 1982). However, the predation pressure imposed by invertebrates on minnow eggs and larvae has probably been under estimated to date (M. Ladle, pers. comm.).

In addition to the considerable body of data available at the River laboratory relating to the River Frome, many other facilities allow the observation of fish behaviour in the wild. In particular the holding channels and fluvarium (figure 3) provide unique opportunities to observe the schooling behaviour of minnows in a near-natural environment.

The fluvarium building at the River laboratory is situated at the site of an old water mill. The building contains two glass sided tanks measuring 6 x 1.5 x 1.5m deep set in parallel into the mill stream (a side branch of the River Frome at East Stoke, plate 2). The entire flow of the mill stream passes through these fluvarium channels. Fish swimming through the channels can be observed in an effectively near natural environment with minimal disturbance. A glass roof allows natural light conditions to prevail. Sluice gates allow water depth and flow rate through each channel to be regulated independently. Mesh screens can also be placed at both up-stream and down-stream ends of the channels so as to keep fish within the fluvarium.

Plate 2. The Mill Stream (a side arm of The River Frome, Dorset) as it enters the IFE fluvarium. Water flow is from the bottom right.

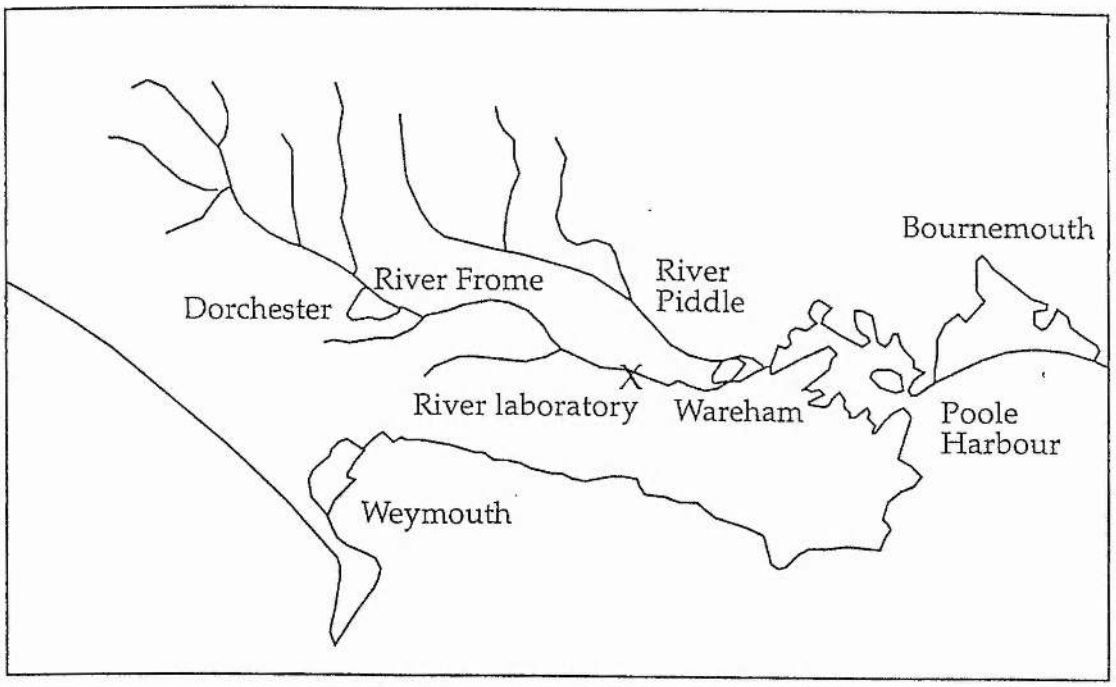

Figure 1. Map showing The River Frome, Dorset, and the position of the IFE River laboratory

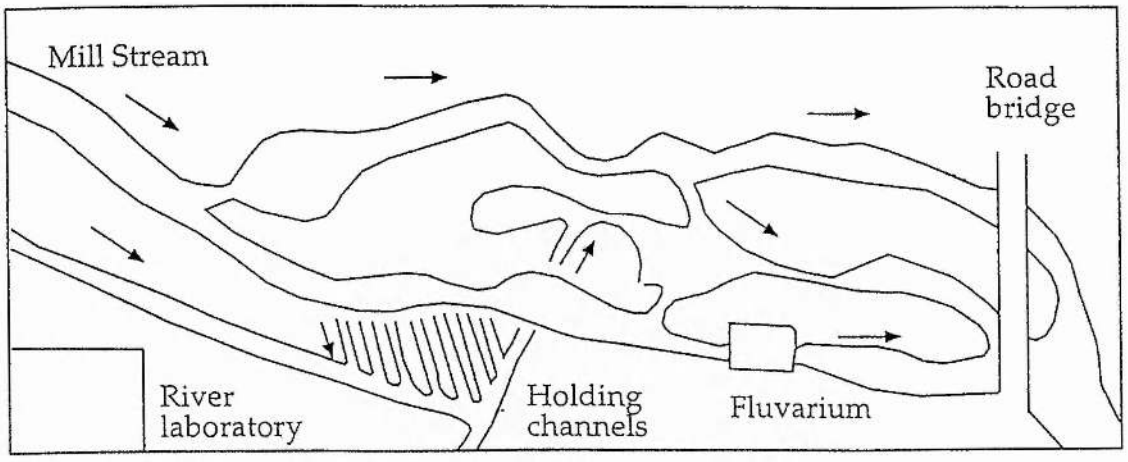

Figure 2. Map of the River Frome and Mill Stream near the IFE River laboratory. Arrows indicate direction of water current through fluvarium and holding channels.

2.2 The Trinidadian guppy

(a) Distribution

The guppy is a member of the family poeciliidae. It is a small teleost fish native to north-eastern South America and the nearby islands of the eastern Caribbean (Rosen & Bailey 1963; Thibault & Schultz 1978). The guppy is abundant and widely distributed through the rivers of Trinidad. However, according to Magurran *et al.* (1995), the guppy populations of most interest are those which live in the northern mountain range of Trinidad (figure 3), because of 2 factors. First, populations there vary significantly in a range of morphological traits. Second, the greater part of this variation can be attributed to one ecological factor, predation pressure. Because these factors clearly link natural selection and adaptation, the Trinidadian river system and associated guppy populations are uniquely interesting and important in the study of evolution in the wild (Magurran *et al.* 1995).

Figure 3 shows the major river systems of Trinidad as well as the sites of some guppy populations exposed to different levels of predation pressure. The guppies of northern Trinidad have been found in allozymic studies (Carvalho *et al.* 1991; Shaw *et al.* 1991) to be separated into two genetically distinct groups. These groups may correspond to the separate colonisation of the two drainage rivers which run from the foothills of the southern slopes of the northern range towards the sea. The Oropuche drainage runs to the Atlantic Ocean whilst the Caroni drainage runs to the Gulf of Paria (see Magurran *et al.* 1995).

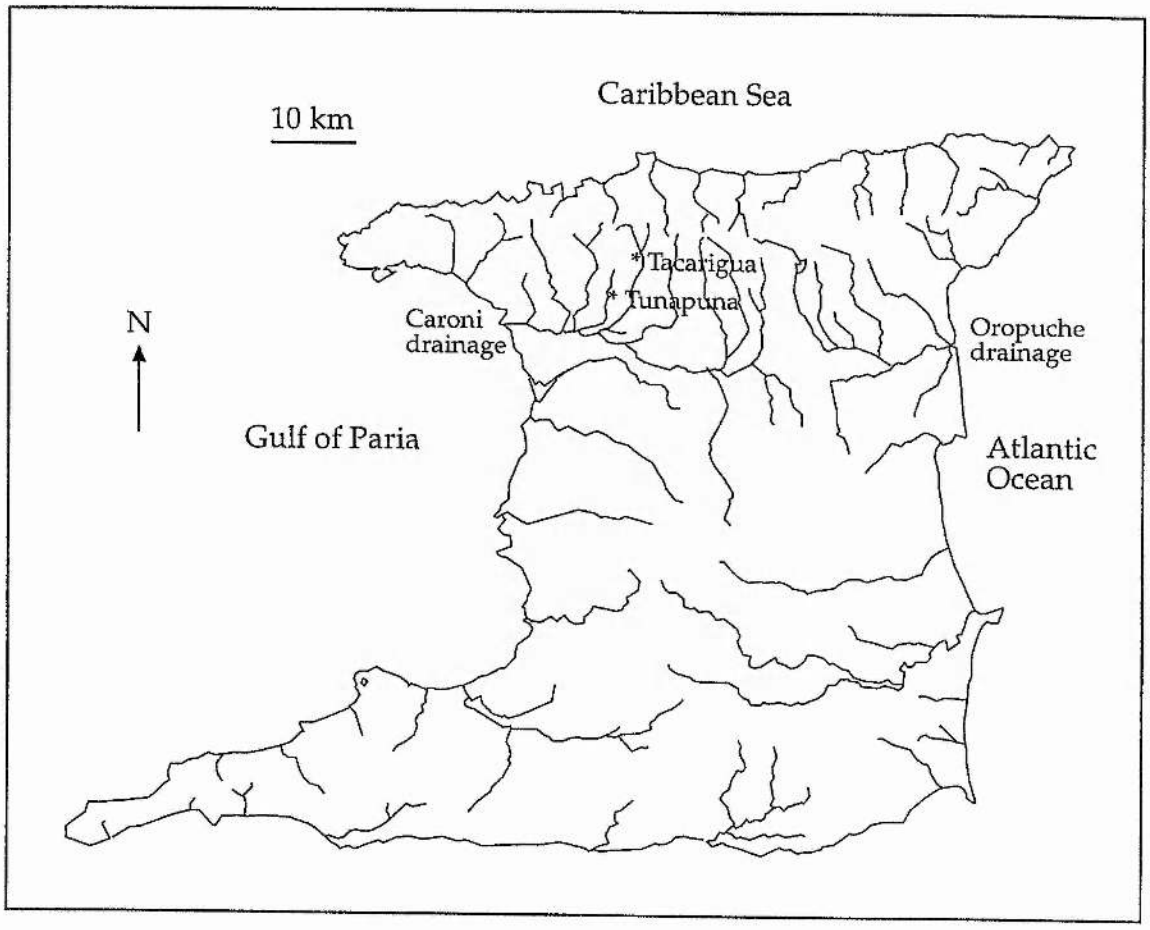

Figure 3. Map of Trinidad showing study sites

The guppy populations present in the streams which drain the north facing slopes are often sympatric with *Macrobrachium*, a genus of freshwater prawn. Endler (1978; 1980; 1983) and Magurran & Seghers (1990a) believe these prawns to be guppy predators, and although Luyten & Liley (1985) disagree, unpublished data (B. H. Seghers & A. E. Magurran pers. comm.) suggest that guppies are indeed subjected to *Macrobrachium* predation in the wild. In the isolated headstreams of the south-facing and north-facing slopes, Hart's rivulus, *Rivulus hartii*, may be present (Rodd & Reznick 1997). This cyprinodont is relatively small in size (maximum standard length = 10.5 cm.), and is a gape-limited omnivore. Nevertheless, gut content analysis (Seghers 1973) showed that 10.2% of 259 *Rivulus* samples included guppies. Although *Rivulus* preys on guppies, it feeds primarily on small immature size classes (Seghers 1973; Liley & Seghers 1975; Seghers 1978; Mattingly & Butler 1994). The extent of the predation pressure to which headstream guppy populations sympatric with *Rivulus* are exposed is small compared to lowland stream populations.

The lowland streams are high predation pressure sites because of the presence of many piscivorous predators in addition to *Rivulus*, including *Crenicichla alta* and *Aequidens pulcher* (cichlidae), 3 species of characin (*Astynax bimaculatus*, *Hemibrycon taeniurus* and *Hoplias malabaricus*) and an eel, *Synbranchus marmoratus*. Prawns in the *Macrobrachium* genus may also be present. All of these species occasionally prey on guppies (Seghers 1973; Liley & Seghers 1975). Piscivores are unable to migrate up many of the streams due to the natural barrier effected by waterfalls (Haskins *et al.* 1961; Liley & Seghers 1975). As a result, the populations of guppies contained within these streams are isolated (or semi-isolated) and are exposed to different intensities of piscivorous predation. This makes the guppy one of the best examples of a single species exposed to variation in predation pressure (Seghers 1973; Seghers 1974b; Liley & Seghers 1975; Endler 1978; Endler 1983; Fraser & Gilliam 1987). It is the differences in communities of predators which accounts for most variation (83%) in guppy life history traits among sites (Reznick & Endler 1982) and only 10% of this variation can be attributed to differences in habitat variables such as stream size (Reznick & Endler 1982). Guppies from *Rivulus* only populations were found by Rodd & Reznick (1997) to usually be larger and more often mature than

populations of guppies that lived in communities with larger predators (where guppies were smaller and more often immature). Because the difference in guppy life-history traits between *Crenicichla* and *Rivulus* dominated populations is consistent for wild caught guppies as well as for their laboratory reared descendants, it is possible to confidently attribute a genetic basis to this difference (Reznick 1982; Reznick & Bryga 1987). The variation in predation regime documented above has been cited as one of the major reasons for the high level of morphological variation in naturally occurring guppy populations (Haskins & Haskins 1951; Haskins *et al.* 1961). Behavioural traits such as schooling tendency (Seghers 1973); anti-predator behaviour (Magurran 1990b; Magurran 1990a) and courtship behaviour such as time spent by males attempting sneaky mating (see below for description of male mating behaviour), (Magurran & Seghers 1994a) as well as female mating preferences (Endler 1983; Kodric-Brown 1985; Houde 1987) also covary with predation pressure (Endler 1995; Magurran *et al.* 1995).

(b) Foraging

Trinidadian guppies are benthic feeders whose diet consist mainly of algae, organic detritus and benthic invertebrates (Dussault & Kramer 1981). In a sample taken from the Upper Aripo population, for example, the proportion of algae and invertebrates in guppy diet was calculated to be 43.5%, 55.2% (males) and 57.1%, 38.4% (females) respectively (Dussault & Kramer 1981).

(c) Reproduction

The guppy is a small livebearing and ovoviviparous (does not nourish its eggs after fertilisation) teleost fish (Thibault & Schultz 1978; Wourms 1981). Broods of young (between 2-18 individuals) are produced by wild females approximately once every month (Reznick & Endler 1982; Reznick & Miles 1989). The different patterns of parental investment and conspicuousness to predators (due in part to the extent of secondary sexual colouration in males) result in strong sexual dimorphism (plates 3 & 4, Liley & Seghers 1975; Abrahams 1993). Female guppies from naturally occurring populations were shown by Seghers (1973) to have a maximum size of 46.5mm compared to the male maximum size of 26.5mm. Females are cryptically coloured, usually beige or olive brown, presumably as a defence against predation (Magurran *et al.* 1995).

Plate 3. Female Trinidadian guppy. Females are cryptic and uniform in appearance

Plate 4. Male Trinidadian guppy with gonopodium swung forward. Male colour patterns are so polymorphic that no two individuals resemble one another

The male anal fin is modified to form an intromittent organ (gonopodium), which allows internal fertilisation to take place. The transfer of sperm can be achieved by either of two possible behavioural sequences: (i) the sigmoid courtship display, which requires the co-operation of the female, and (ii) gonopodial thrusting (sneaky mating attempts, Clark & Aronson 1951; Kadow 1954; Baerends *et al.* 1955; Liley 1966; Luyten & Liley 1985; Endler 1987). The brightly coloured males display throughout the female ovarian cycle, although females are only receptive for a few days during this time (whilst virgins and for the one or two days immediately after giving birth). Female guppies can store sperm and are able to produce broods for many months after only a single mating (Winge 1937). The extent of guppy sexual behaviour was documented by Farr & Herrnkind (1974) who counted approximately 7 sigmoidal displays within a 5 minute period in a laboratory study. A similarly high rate of 13 displays per male per 5 minutes was found by Farr (1975) in a natural population. The rate of gonopodial thrusts in the same natural population was found to be between 0.5 and 3.0 thrusts per male per 5 minutes (Farr 1975), and Magurran & Seghers (1994c) noted rates as high as 1 thrust per minute.

Current interest in the extent to which male gonopodial thrusting undermines female choice is intense, especially because male guppies can adopt either mating strategy (gonodpodial thrusting or sigmoid display). Recent work has demonstrated that males undertaking high rates of sneaky mating also have high reserves of sperm (Matthews *et al.* 1997). Since there is evidence for multiple paternity in guppy broods (Winge 1937; Hildemann & Wagner 1954; Haskins *et al.* 1961) it will be interesting to see if future work reveals the extent to which gonopodial thrusting contributes to brood paternity.

CHAPTER 3

The partner choice decisions of free-swimming female guppies.

3.1 Introduction

The benefits to individuals of group living have been demonstrated for many animals (Bertram 1978; Pulliam & Caraco 1984). For example, ostriches, *Struthio camelus*, gain foraging benefits by flocking because maintaining vigilance for approaching predators becomes a task shared by all group members, allowing each individual more time on average for feeding (Bertram 1980). Increased foraging and anti-predator benefits are also the two main functions of fish schooling behaviour (Pitcher & Parrish 1993). For example, schools of minnows, *Phoxinus phoxinus*, and goldfish, *Carassius auratus*, (Pitcher *et al.* 1982) as well as stone loach, *Noemacheilus barbatulus*, (Street & Hart 1985) find food faster than solitary individuals. As school size increases, however, the benefit of speedy discovery of a food patch is balanced by the cost of competing for resources with many other school-mates (Bertram 1978; Street *et al.* 1984). Similarly, the cost of increased conspicuousness to a predator may be conferred upon individual fish as school size increases, although this depends on visibility and water quality (Murphy 1980). Reviews of the anti-predator effects of group-living are provided by Neill (1974); Pulliam & Caraco (1984); Godin (1986; 1997); Turner & Pitcher (1986) and Pitcher & Parrish (1993).

Not only do individuals enjoy the passive advantages of group living, risk of attack decreasing as a function of increasing school size (Bertram 1978; Foster & Treherne 1981), but fish exhibit many behavioural responses which include amongst others, the confusion effect (Milinski 1977), encounter-dilution effect (Turner & Pitcher 1986), early predator warning (Magurran *et al.* 1985), many predator evasion manoeuvres (Pitcher & Parrish 1993) and predator inspection behaviour (Pitcher *et al.* 1986a).

The efficiency with which some of these anti-predator behaviours are undertaken is increased even further if the fish are familiar with one another (Chivers *et al.* 1995). For example, schools of (familiar) fathead minnows, *Pimephales promelas*, taken from the wild are more cohesive, increasing the amount of dashing and predator inspection behaviour, and decreasing the amount of freezing behaviour when exposed to predatory threat compared with schools comprised of unfamiliar individuals (Chivers *et al.* 1995). This suggests that fish in the wild should gain significant advantages by joining a familiar school of conspecifics in the event of a predatory attack. Furthermore, it is also known that individuals choosing to school with familiar conspecifics gain foraging benefits by doing so (Metcalfe & Thomson 1995). It is predicted that fish may also gain anti-parasite benefits from associating with familiar conspecifics. If information regarding the parasite load of school-mates can be obtained during the development of familiarity, then, the opportunity arises for preferential association (within each school), with a nearest neighbour of low parasite load. Discrimination between fish on the basis of parasite load is known to be possible for threespine sticklebacks, *Gasterosteus aculeatus*, (Dugatkin *et al.* 1994). In a laboratory choice test juveniles avoided parasitised conspecifcs on the basis of parasite presence as well as the effect of parasites on stickleback behaviour, and in doing so it was suggested that they gained anti-parasite benefits (Dugatkin *et al.* 1994). Individuals may also be afforded the benefits of decreased aggression levels as a result of the formation of a stable dominance hierarchy between familiar individuals (Getty 1989). Furthermore, the benefits of co-operative alliances (Dugatkin & Wilson 1992) may be conferred upon individual members of familiar schools, as recognition and familiarity are pre-requisites of co-operation.

Many freshwater fish including bluegill sunfish, *Lepomis macrochirus*, (Brown & Colgan 1986), threespine sticklebacks (Van Havre & FitzGerald 1988), fathead minnows (Brown & Smith 1994) and guppies, *Poecilia reticulata*, (Magurran *et al.* 1994) demonstrate a preference for familiar conspecifics when given a choice of schooling partners. Indeed, the role of familiarity in the recognition abilities of fish species may have been underestimated to date. Evidence to support this hypothesis comes partly from investigations of the homing tendency of anadromous fishes. Experiments which have demonstrated preferences for related conspecifics by fish, include those on Atlantic

salmon parr, *Salmo salar*, (Stabell 1982; Stabell 1987; Moore *et al.* 1994); Baltic salmon parr, *Salmo salar*, (Folke *et al.* 1992); coho salmon, *Oncorhynchus kisutch*, (Quinn & Tolson 1986); juvenile Arctic charr, *Salvelinus alpinus*, (Olsén 1986); sockeye salmon, *Oncorhynchus nerka*, (Groot *et al.* 1986); and guppies (Warburton & Lees 1996). However, these may not have controlled appropriately for the possible effects which common rearing regimes have on the development of familiarity within groups (but see Quinn & Busack 1985, for an exception). For example, Warburton & Lees (1996) recently found that domestic guppies seem to prefer schooling with related conspecifics. However, the possibility that familiarity was affecting these schooling decisions could not be rejected since the kin groups had been raised together for one week before the trials were undertaken.

Although the importance of familiarity in the schooling decisions of fish is clear, little is understood of how the schooling preferences demonstrated under laboratory conditions are related to the partner choice decisions of free-swimming individuals in the wild. Laboratory studies have often given individual fish a choice of schooling with either of two 'schools' of conspecifics held at either end of an aquarium (e.g. Van Havre & FitzGerald 1988; Dugatkin & Wilson 1992; Magurran *et al.* 1994). Only a few studies have investigated school fidelity in the wild. Helfman (1984) observed the movement and behaviour patterns of 102 individually identifiable yellow perch, *Perca flavescens*, in Cazenovia Lake, New York. Although individual fish did co-occur, this was found to be a function of schooling tendency rather than school fidelity (Helfman 1984).

Similarly, in a census of bluegill sunfish from North American lakes, Wilson *et al.* (1996) established that individual fish were predictably found to occupy either littoral zone or open water habitats. High parasite load was used as an indicator of long term use of the littoral zone. These preferences could not be attributed to availability of prey items in each habitat because fish body shape (which reflects dietary preference) was not consistent across habitats. Although bluegill sunfish from open water are usually more fusiform with smaller pectoral fins than individuals collected from the littoral zone (Ehlinger & Wilson 1988), Wilson *et al.*'s findings were reached irrespective of differences in morphology (Wilson *et al.* 1996). Is it therefore possible

that the co-occurence of bluegill sunfish reflects a preference for particular conspecifics rather than habitat preference? Wilson (1998) suggests that a simpler explanation, involving the theory of density dependence, is possible. Preference for one location is likely to simply be a function of how many other individuals in the population are occupying the two habitat types already, because competition for resources increases with increasing group size (Wilson *et al.* 1996).

This experiment aims to investigate whether female guppies allowed to swim freely amongst conspecifics choose familiar school-mates. The null hypothesis being tested is that schools are composed of random assemblages of individuals. In addition, because fish are expected to gain anti-predator advantages by joining a school of familiar conspecifics the role which predator threat plays in choice of schooling partner will be investigated.

3.2 Methods

This experiment was conducted during November 1995. The study animals were normally held as large breeding stocks in the laboratory, and were descendants of guppies collected from the wild from the Lower Tacarigua River, Trinidad. Thirty-six females of a similar size (mean total length = 31.8 ± 1s.e. 0.58mm) were removed from the stock tanks and housed in an aquarium for four days to allow complete mixing before separating them into six groups of 6 fish each. This ensured that the fish were equally familiar at the beginning of the experiment. Female guppies have been chosen as suitable experimental animals because they have a higher schooling tendency than males, and are cryptically coloured, unlike males which can be individually identified on the basis of colour patterns (plates 3 & 4). Each group of fish was allocated its own aquarium (45 x 32 x 32 cm deep) so that the groups were isolated visually and olfactorily from one another. Each tank contained a water filter, air stone and clump of Java moss, *Vesicularis dubyana*. The fish were fed daily with Tetramin™ and kept at 25°C on a 12 h light regime. Fish which shared a tank were designated 'familiar' fish whereas conspecifics in different tanks were known as

'unfamiliar' fish. The fish were housed together for 2 weeks before the trials began.

(a) Experiment

Two days before the trials began, fish from each tank were given a small alcian blue batch mark on their upper or lower caudal peduncle. This meant that familiar females could be easily distinguished from the unfamiliar fish. A test aquarium (90 x 32 x 20 cm water depth) was used as the observation arena. A clump of java moss was placed at one end of the aquarium, and marks drawn onto the glass divided the experimental arena into 3 sections for observational purposes. Three equally sized familiar females, randomly chosen from one of the six tanks and 3 equally sized unfamiliar female guppies, from a different tank, were gently transferred to the test aquarium. A focal familiar fish from each group of 3 was observed in turn for 15 min before and after a predator model was introduced. The model (14cm long) resembled *Crenicichla alta,* a naturally occurring pike cichlid predator of guppies in the wild (see Magurran & Seghers 1990a). The model could move a little in the water column despite being anchored to a weight by a monofilament line (figure 4). The guppies did not seem to be disturbed by the careful and slow approach of the observer to the experimental tank in order to introduce the model.

The time that each focal fish spent schooling with a familiar or unfamiliar nearest neighbour during these two periods was measured. Total schooling time was also recorded. For the purposes of this experiment a focal fish was defined as schooling if it was within 3 body lengths of another female. This can be regarded as a conservative definition of schooling as Pitcher (1983) considered 4 body lengths to be an appropriate distance by which school members would be maximally separated, while Magurran *et al.* (1992) used 5 body lengths in a study of guppies in the wild. At the end of the trial the total length of each fish was measured in order to confirm that fish size did not vary significantly between tanks. Observations were made on one fish from each tank per day, so that over the period of three days, three fish from each tank were tested (n = 18). No fish was tested more than once. The position of the predator model and Java moss was alternated randomly.

(b) Control

The aim of the control experiment (conducted during December 1995) was to ascertain whether the marking procedure influenced schooling preferences. Six familiar guppies, (not previously used as experimental animals) were removed from a stock tank and placed together in an aquarium (45 x 32 x 32 cm deep). Three randomly selected individuals were given one batch mark (alcian blue dye on the upper caudal peduncle) and the remaining three were marked differently (alcian blue dye on the lower caudal peduncle). After a two day recovery period the fish were tested for their nearest neighbour preferences in the experimental aquarium for 15 minutes before and after a predator model was introduced, as described above. The trial was repeated four times so that observations were made on eight individuals in total. After 6 days, the alcian blue dye had faded beyond the level at which individual fish could be distinguished.

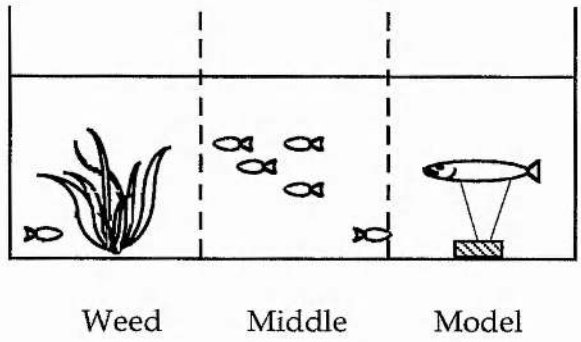

Figure 4. Schematic representation of experimental tank (not to scale). Hatched lines denote the divisions (marked with ink on the aquarium glass for ease of observation) between the three sections of the tank ('weed', 'middle' and 'model'). Other features illustrated include a clump of java moss, predator model, and 6 female guppies. Three guppies were familiar to one another (having previously been housed together for 2 weeks) but were unfamiliar to the remaining 3 guppies and *vice versa*. The nearest neighbour preferences of focal female guppies (for familiar and unfamiliar school-mates) were measured before and after the introduction of the predator model in order to investigate two questions. Do females guppies preferentially associate with familiar school-mates? Does predator threat play a role in choice of schooling partner?

(c) Data analysis

The total number of guppies in each trial was relatively small (3 familiar + 3 unfamiliar). Thus the ratio of familiar to unfamiliar fish became highly skewed when one individual was chosen as a focal fish: becoming biased towards unfamiliar conspecifics. Preferences for the remaining 2 familiar and 3 unfamiliar schooling partners were therefore expressed as time (s) spent schooling per familiar and per unfamiliar fish (partner choice data). Preferences for the three sections of the aquarium tank were expressed as time (s) spent by focal fish in each section (distribution data). A three-way ANOVA investigated the effect of familiarity, threat and group (aquarium tank) on partner choice during schooling. A second three-way ANOVA investigated the effect of section, threat and group (tank) on distribution of the focal fish.

3.3 Results

The analysis confirmed the hypothesis that female guppies prefer to school with familiar individuals over unfamiliar conspecifics ($F_{1,48}$ = 25.85, $P < 0.001$, table 3, figure 5). Contrary to expectations, however, threat had no effect on partner choice decisions ($F_{1,48} = 0.06$, $P = 0.812$, table 3, figure 5). Interaction terms were not significant (table 3).

Female guppies had an overall preference for the section of the aquarium tank containing weed ($F_{2,72} = 5.65$, $P < 0.01$, table 4), spending on average 43% of total time in this section of the experimental tank. Threat had no effect on guppy distribution ($F_{1,72} = 0.00$, $P = 0.999$, table 4). However, the interaction between section and threat was significant ($F_{2,72} = 18.73$, $P < 0.001$, table 4, figure 6). Guppies spent less time in the 'model' section of the experimental tank when the model predator was introduced, and were observed to hide under the weed at the opposite end of the tank during this period, demonstrating that the guppies viewed the model as a threat. Other interaction terms were not significant (table 4).

The control study showed that schooling decisions were not influenced by the marking procedure. Female guppies equally familiar

with one another did not demonstrate a preference for schooling with individuals of same or different batch mark to themselves (time per nearest neighbour: one-way ANOVA $F_{1,6} = 0.07$, $P = 0.802$). There is no reason to suspect, therefore, that the preferential association with familiar fish found in the main experiment can be attributed to preferences for, or avoidance of, alcian blue batch marks.

Table 3. Three-way ANOVA of female guppy schooling behaviour, measured as the mean time spent schooling (s) per familiar or unfamiliar stimulus fish. The factors are familiarity, threat and group (tank). * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	29017	25.85	0.000
Threat	1	64	0.06	0.812
Group	5	408	0.36	0.871
Familiarity * threat	1	796	0.71	0.404
Familiarity * group	5	1007	0.90	0.490
Threat * group	5	246	0.22	0.952
Familiarity * threat * group	5	1468	1.31	0.277
Error	48	1122		

Table 4. Three-way ANOVA of female guppy schooling behaviour, measured as the total time spent (s) in each section of the aquarium tank. The factors are section, threat and group (tank). * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Section	2	84936	5.65	0.005
Threat	1	0	0.0	0.999
Group	5	7731	0.51	0.765
Section * threat	2	281627	18.73	0.000
Section * group	10	29571	1.97	0.050
Threat * group	5	8275	0.55	0.738
Section * threat * group	10	24908	1.66	0.108
Error	72	15037		

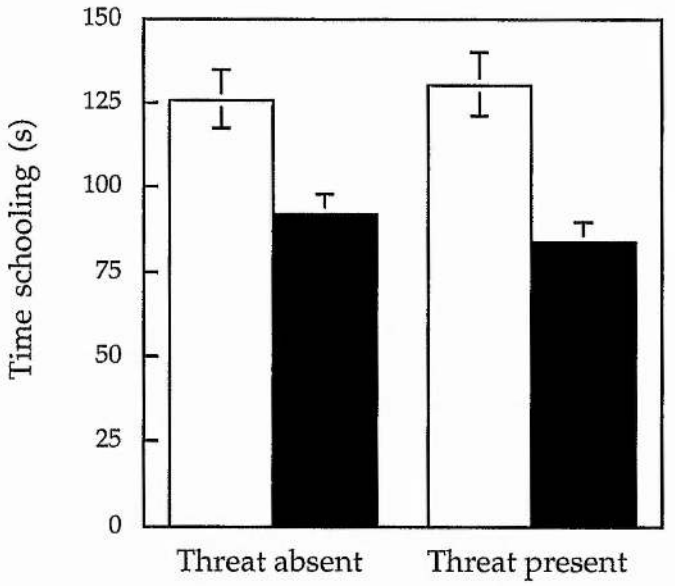

Figure 5. Nearest neighbour preferences of female guppies in terms of the time schooling per familiar (❑) and unfamiliar (■) fish. Data are given as means ± 1s.e. (n=18) before and after the introduction of a predator model.

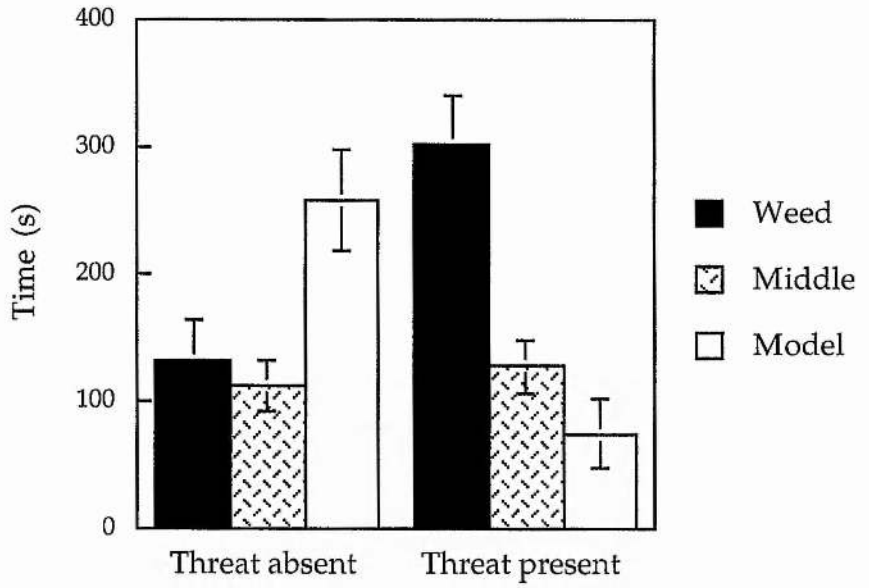

Figure 6. Distribution of guppies between the three sections of the experimental aquarium, measured as total time in each section. Data are given as means ± 1s.e. (n=18) before and after the introduction of a model predator.

3.4 Discussion

This investigation confirms that female guppies prefer to school with familiar over unfamiliar conspecifics. Not only do these fish prefer school-mates with whom they have prior experience, but females allowed to swim freely amongst conspecifics also choose familiar nearest neighbours, that is fish with whom they have previously been housed for 2 weeks. It seems that guppies are able to discriminate between conspecifics, and furthermore that individuals which recognise one another may form sub-groups within schools by swimming next to one another. These results suggest that fish schools in the wild may not be composed of random assemblages of individuals, rather that school membership is predictable.

Magurran *et al.* (1994) have previously shown that guppies descended from the Lower Aripo and Lower Turure River populations prefer familiar conspecifics i.e. those with whom they had previously been housed for 2 months. Guppies living at these sites are subject to high predation pressure, notably from the pike cichlid, *Crenicichla*, (Magurran & Seghers 1991), as are the Lower Tacarigua guppies used in this experiment. Indeed, Haskins *et al.* (1961) suggested that *Crenicichla* might be a specialised guppy predator and more recently Endler (1987) has reported observations he made on this predator attacking guppies in the wild. Although fish remains were found in over 50% of the *Crenicichla* stomachs analysed by Seghers (1973), mollusc and insect remains were also found. Other freshwater fish species which demonstrate a preference for familiar school-mates include bluegill sunfish (Brown & Colgan 1986; Dugatkin & Wilson 1992), threespine sticklebacks (Van Havre & FitzGerald 1988), and fathead minnows (Brown & Smith 1994).

The advantages to fish of recognising and preferentially associating with familiar conspecifics include among others, the foraging benefits of being able to discriminate between conspecifics of known competitive ability (Metcalfe & Thomson 1995). Foraging benefits may also become available to fish which associate with known conspecifics in order that stable dominance hierarchies may be

maintained. Both dominant and subordinate individuals are thought to benefit from stable groups because while dominants tolerate subordinates (which would otherwise be excluded from feeding territories), they are also able to displace them in order to obtain food (Rohwer & Ewald 1981; Ydenberg *et al.* 1988; Senar *et al.* 1990). Getty (1989) and Ydenberg *et al.* (1988) have suggested that familiar individuals benefit from hierarchical relationships because they are therefore able to avoid costly contests with many opponents. However, Olsén *et al.* (1996) were unable to find any effect of familiarity on aggression in brown trout, *Salmo trutta*.

This study has also shown that schooling decisions are not affected by risk of predation although it had been predicted that time spent with familiar school-mates would have increased as a result of increased predator threat. Preferences for familiar schooling partners were not increased even when a model predator was presented to the guppies. The model predator used in this experiment was the same one used by Magurran & Seghers (1994b) during field work in Trinidad and by Magurran *et al.* (1992; 1995) during laboratory studies of guppies under predation pressure. Dugatkin & Godin (1992) have also successfully used a predator model during observations of guppy anti-predator behaviour. In the wild, guppies from 9 populations (of which 5 co-occur with *Crenicichla*) responded with appropriate anti-predator behaviour, that is predator inspection, when presented with this realistic threat (Magurran & Seghers 1994b). Guppies from the Lower Tacarigua population were among those found to react to the presence of the model by increasing the time spent schooling and decreasing the closest approach distance to the model (Magurran & Seghers 1994b). It is therefore unlikely that the guppies in this experiment did not perceive the model as a predatory threat. The use of models in behavioural studies such as this avoids the ethical problems of placing fish under true threat of predation (Huntingford 1984).

Another explanation for guppy schooling preferences remaining unchanged despite increased predator risk may be due to the relatively low stage of escalation of the apparent threat in the predators' attack sequence. Endler (1986) describes a predation event as being composed of five stages including detection, identification, approach, subjugation and consumption of prey. The presentation of the model predator may

have simulated the detection stage (where a prey item is recognised as distinct from its background) and possibly even the identification stage of a predatory event (when the predator decides whether the prey item is edible or not). However, the model was not made to approach the guppies. The anti-predator defence mechanisms applied by fish to counter the detection and identification stages include crypsis and mimicry (Endler 1986). It is possible that only approach or subjugation stages would elicit a behavioural response such as increased preference for familiar conspecifics. Perhaps the preference of guppies for their familiar school-mates would be further enhanced only if they were presented with, for example, a model predator mimicking the stalking approach behaviour of a live piscivore or a model apparently launching an escalated attack. It would also be interesting to investigate any inter-population differences in schooling decisions. Fish from high predation habitats such as the guppies in this experiment are under a greater selection pressure to present better anti-predator strategies than guppies from low predation habitats. If schooling with familiar fish increases school cohesion as well as other anti-predator behaviours (Chivers *et al.* 1995), then perhaps guppies from a high predation population routinely demonstrate a bias towards familiar school-mates. Similarly, it may be less risky to routinely school with familiar school-mates than to leave the safety of a school in search of a familiar conspecific only when a predator is detected and a predation event may already be in progress.

The association of familiar fish within groups suggests naturally occurring schools to be likely places in which the formation of co-operative alliances between individuals might be found. For co-operation between individuals to evolve, either non-random interactions (Hamilton 1964) or conditional behaviours which result in individuals acting co-operatively mainly to other co-operators (Axelrod & Hamilton 1981) are required. Schooling is one example of non-random associations of individuals. See Dugatkin (1997) for a concise overview of co-operation in many animal taxa. The formation of co-operative alliances has been shown to confer foraging benefits upon yellowtail, *Seriola lalandei*, which hunt jack mackerel, *Trachurus symmetricus*, (Major 1978). Yellowtail exploit the schooling behaviour of the mackerel by herding a school against a shore line. Yellowtail rush through the trapped mackerel school and individual mackerel then become separated, making it easier for yellowtail to catch their prey

(Schmitt & Strand 1982). Co-operative foraging is also known to occur between bluegill sunfish (Mittlebach 1984; Dugatkin & Wilson 1992). Bluegill sunfish were allowed to forage in pairs, and their foraging success was measured by counting the number of mealworms remaining at the end of each trial. Each bluegill sunfish was paired with each of 5 tank-mates in a series of trials. In subsequent two-choice tests, bluegill sunfish preferentially associated with individuals with whom they had successfully foraged in the past, suggesting that these fish can distinguish between co-operative and non co-operative partners on the basis of foraging cues (Dugatkin & Wilson 1992).

Co-operative anti-predator behaviour has been observed in many freshwater fish. Indeed the phenomenon of predator inspection behaviour where a small number of fish separate from a school and approach a potential predator (Pitcher *et al.* 1986) has provided an ideal opportunity for the study of co-operation, particularly reciprocity and the tit-for-tat strategy (Milinski 1987; Dugatkin 1988). Predator inspection behaviour is costly to individual fish who suffer increased risk of predation (Magurran & Girling 1986; Dugatkin 1992) or sneaky mating attempts in the case of female guppies (Magurran & Nowak 1991) compared to fish who remain behind in the main body of the school. However, the benefits available to these individuals must outweigh the costs if the immediate risk of being eaten can be avoided. The question remains, however, of which of the inspectors leads during the inspection, as risk of being eaten is greatest for the fish nearest the predator (Dugatkin 1992). It was Milinski (1987) who first suggested that pairs of inspecting fish may be trapped in a prisoner's dilemma and that pairs of fish co-operate by employing the tit-for tat strategy (Milinski 1987; Dugatkin 1988) during predator inspection in answer to this problem. See also Dugatkin (1997) for an overview of tit-for-tat and co-operation during predator inspection behaviour. It is hoped that future work may elucidate the role which familiarity plays in the formation of these co-operative alliances. It is predicted that familiar fish perform better inspection behaviour than unfamiliar fish. By trusting an inspection partner enough to maintain the inspection and therefore gain more information about the 'state' of the predator, benefits are expected to accrue to these familiar fish.

CHAPTER 4

The effect of predation risk on partner choice decisions in the European minnow[1]

Evidence that fish have the cognitive ability to discriminate among conspecifics, even to the extent of choosing particular school-mates, is now well documented (e.g. Milinski *et al.* 1990b; Dugatkin & Wilson 1992; Dugatkin & Wilson 1993). There is strong evidence for the ability of fish to make partner choice decisions on the basis of familiarity, for example threespine sticklebacks, *Gasterosteus aculeatus*, (Van Havre & FitzGerald 1988); bluegill sunfish, *Lepomis macrochirus*, (Brown & Colgan 1986); fathead minnows, *Pimephales promelas*, (Brown & Smith 1994) and guppies, *Poecilia reticulata*, (chapter 3, table 2 and Magurran *et al.* 1994). In the case of guppies, Magurran *et al.* (1994) demonstrated that females housed together for two months under standard laboratory conditions were subsequently capable of distinguishing familiar tank-mates from unfamiliar fish. The preference for familiar schooling partners has also been observed for wild fish in the laboratory (Dugatkin & Wilson 1992; Brown & Smith 1994) and in the wild (chapter 7, Griffiths & Magurran 1997b). In the former example, Brown & Smith (1994) found that wild fathead minnows from Pike Lake, Saskatchewan, preferentially associate with familiar conspecifics, that is members of their naturally occurring schools. This schooling preference is even demonstrated by fish (e.g. Bluegill sunfish) when test fish are visually or chemosensorily isolated from stimulus fish (Dugatkin & Wilson 1992).

Fish may prefer to school with familiar individuals for more than one reason. One possibility is that there may be foraging advantages of schooling with familiar conspecifics (Dugatkin & Wilson 1992; Metcalfe & Thomson 1995). Indeed, European minnows, *Phoxinus phoxinus*, do have the ability to recognise poorer foraging competitors, and in future encounters preferentially school with these individuals in

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1Data from part of this study have been published as Griffiths, S. W. (1997) Preferences for familiar fish do not vary with predation risk in the European minnow. Journal of Fish Biology, 51, 489-495.

order to gain foraging advantages (Metcalfe & Thomson 1995). This might explain the preference for familiar school-mates even by fish naturally exposed to low predation pressure (chapter 7, Griffiths & Magurran 1997b). It is also possible that schooling decisions may be made on the basis of a range of characteristics (for example, morphology, anti-predator behaviour, or competitive ability), so that an individual may trade-off the costs and benefits of choosing one schooling partner in preference to other fish with which it is familiar. It is known that schools of familiar fathead minnows demonstrate better anti-predator behaviour than schools of fish unfamiliar to one another (Chivers *et al.* 1995). Chivers and his colleagues measured a range of anti-predator responses (increased school cohesion, dashing, number of predator inspections and decreased freezing behaviour) in schools of familiar fish exposed to predatory threat. Although the anti-predator and foraging benefits associated with schooling with familiar fish are clear (Chivers *et al.* 1995; Metcalfe & Thomson 1995), the question of whether preference for familiar conspecifics is mediated by predatory threat remains unanswered.

This chapter describes two experiments which were undertaken in the near-natural conditions of a fluvarium, using European minnows. This fish (a member of the cyprinid family) is an ideal species for the study of schooling behaviour, occurring in discrete schools and being numerous in many rivers and streams throughout Europe. In the River Frome, Dorset, minnows co-occur with piscivores of which the pike, *Esox lucius*, is the most renowned and imposes a high predation pressure (Mann 1982). Both studies aimed to answer the following questions in order to test two hypotheses: do minnows choose familiar over unfamiliar school-mates; and does preference for familiar schooling partners increase with risk? In the first experiment an overview of school composition was obtained and a live pike used in order to present the minnows with a predatory threat. In the second experiment, however, partner choice decisions of individual fish were measured and a realistically painted predator model was used to simulate threat. It was hoped that remote control of the model pike would increase the realism of the apparent predatory threat by mimicking the stalking behaviour of a live pike preparing to launch an attack (Neill & Cullen 1974). Comparison of the results of these two experiments will address the question of whether a model adequately

mimics a live piscivore and, moreover, whether in future studies of this kind use of live piscivores can be justified.

4.1 Do preferences for familiar minnows vary with risk of predation from a live pike?

Introduction

Recent work has demonstrated that many species of freshwater fish preferentially associate with familiar conspecifics (Van Havre & FitzGerald 1988; Magurran *et al.* 1994) - table 2. However, studies of the schooling decisions of wild fish in the wild are rare. This experiment aims to investigate the schooling preferences of the European minnow in the near-natural conditions of a fluvarium in the River Frome, U.K. The anti-predator benefits afforded to individual members of schools of familiar fish (Chivers *et al.* 1995) suggests that preference for familiar school-mates will be increased by the threat of predatory attack. Minnow schooling behaviour was observed in the absence and presence of a live pike.

Methods

The experiment was undertaken between July 21 and August 15 1995 using a fluvarium as described in chapter 2. During trials fish were restricted to a section of one fluvarium channel measuring 4m long x 60cm wide x 40cm water depth. Water (mean temperature of 21.2°C ± 1s.e. = 0.27) flowed over a 40cm layer of gravel (mean diameter 2cm) at a mean velocity of $0.13ms^{-1}$ ± 1s.e. = 0.02. The fluvarium's glass roof allowed observations to be made under natural light conditions. The channel was subdivided for observation purposes into three sections along the horizontal axis of the fluvarium (upstream, midstream and downstream) and two sections along the vertical axis of the fluvarium (top and bottom of water column) by marks on the glass walls visible to the observer. A clump of the macrophyte, *Ranunculus penicillatus* ssp. *pseudofluitans*, was placed in the downstream section and a cage

containing a pike (details below, figure 7) was located in the upstream section.

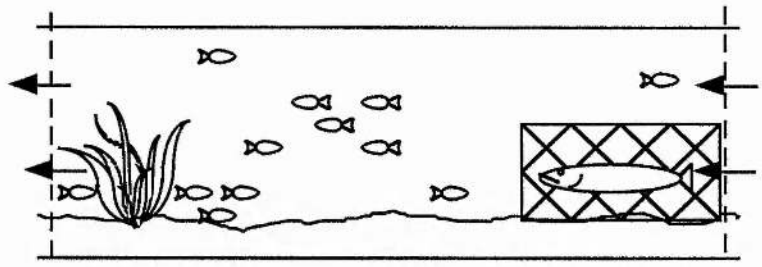

Figure 7. Schematic representation of fluvarium channel (not to scale). Arrows indicate direction of water flow and pike in cage is positioned upstream. Other features illustrated include a clump of weed, layer of gravel and two screens which prevent the escape of the 12 minnows (6 from each of 2 schools) from the observation arena. The position and composition (familiar and unfamiliar fish) of focal minnows' schools was observed in the absence or presence of the caged pike.

(a) Experiment

Schools of (approximately 50) minnows were obtained from each of two sites (8 km apart) on the River Frome, Dorset by a combination of seine netting and electrofishing. Members of the same school were defined as 'familiar' whereas fish from different schools and different sites were defined as 'unfamiliar' to one another. These schools were housed in visual and olfactory isolation in separate cages (in side channels of a tributary of the River Frome, see chapter 2) for at least 3 days before the beginning of the experiment. The fish were fed twice daily with ground Promin™ commercial fish food. A pike was obtained by electric fishing a stretch of the River Frome. This fish was then retained in a side channel of a tributary of the River Frome (downstream from the caged minnows), and fed *ad libitum* with recently killed dace, *Leuciscus leuciscus*. Trials were conducted either in the presence or absence of the pike. In the 'pike-present' trials the pike (total length 40 cm) was housed in a large metal cage (80 x 45 x 27 cm, figure 7) situated in the upstream section of the fluvarium channel. The pike was placed in this cage 1 hour before the minnows were introduced to the fluvarium. The cage mesh diameter was small enough to prevent the pike from striking at minnows, whilst being large enough to allow minnows to enter. In the

'pike-absent' trials, the cage was empty but remained in the upstream section of the fluvarium channel.

Before a trial six minnows from each of two schools were measured (total length) and each individual given a batch mark. This consisted of a spot of Radiant® fluorescent pigment applied to each fish's caudal fin using a small paint brush (plate 5). All 12 fish were then immediately placed into the fluvarium and allowed to settle for 5 hours. During each trial, observations were made on a focal fish from each group of 6 for 15 min. Observations of schooling behaviour were undertaken from behind a hide to avoid disturbance to the fish. Time spent by the focal fish in each of the (upstream, midstream and downstream) sections of the channel was measured. The proportion of the focal fish's school composed of familiar and unfamiliar conspecifics was recorded every 30 s. In addition, the time spent by focal fish in the top and bottom halves of the water column and the number of bites taken by focal fish at gravel, wire mesh of screens, weed, water surface and in the water column was measured. No fish was used more than once and 20 trials were completed in total.

(b) Data analysis

Preferences for familiar and unfamiliar schooling partners were expressed as percentage of total number of fish in a school (school composition data). Preferences for fluvarium section (upstream, midstream and downstream) were expressed as percentage of total time spent in each (distribution data). Proportion data were used in order to account for variation in schooling tendency between schools. A two-way ANOVA was conducted to test the effects of familiarity and threat on school composition while a second two-way ANOVA investigated the effects of section (upstream/midstream/downstream) preference and threat on minnow distribution. All proportion data were arcsine transformed for analysis (Sokal & Rohlf 1995).

Plate 5. European minnows marked with Radiant™ fluorescent pigment

Results

Minnow schools were found to be composed of significantly more familiar than unfamiliar fish ($F_{1,76}$ = 18.09, P < 0.001, table 5), familiar individuals comprising 59% of the mean school size of 7.9 individuals (± 1s.e. = 0.5). The mean proportion of schools composed of familiar fish differed significantly from 50% (t_{40} = 3.04, P = 0.0042). School composition was unaffected by the presence of a live pike ($F_{1,76}$ = 0.00, P = 1.00, table 5, figure 8). Focal fish preferred familiar school-mates equally well in the presence or absence of the pike. Interaction effects were non significant (table 5).

Minnows demonstrated an overall preference for the upstream portion of the fluvarium channel (proportion of total time spent in each section: $F_{2,114}$ = 27.83, P < 0.001, table 6, figure 9). Of the total time available, minnows spent on average 60% in the upstream section containing the pike cage. Perhaps the abundance of water borne food items was greatest at this point. Threat had no effect on time budget data (proportion of total time spent in each section: $F_{1,114}$ = 0.03, P = 0.855, table 6) nor on mean school size (one-way ANOVA, $F_{1,38}$ = 0.16, P = 0.687). However, the interaction effect between threat and section was significant ($F_{2,114}$ = 7.44, P < 0.01, table 6, figure 9). Time spent by minnows in the upstream section was reduced when the pike was present suggesting that the pike was identified by the minnows as a potential threat (figure 9).

Minnows spent most time (74%) in the bottom half of the water column nearest the gravel substratum (proportion of total time: two-way ANOVA, $F_{1,76}$ = 28.21, P < 0.001). Vertical distribution of fish was not affected by threat ($F_{1,76}$ = 0.00, P = 0.983), and the interaction effect was not significant ($F_{1,76}$ = 2.49, P = 0.119). The amount of food taken by minnows was significantly affected by position from which the food particles could be obtained (one-way ANOVA, proportion of total number of bites: $F_{3,132}$ = 35.95, P < 0.001). The majority of food (70% of bites) was obtained from the water column. Sixteen percent of bites were made at the upstream mesh screen, while the remainder of the foraging behaviour was observed either at the water surface or on the gravel bottom.

Table 5. Two-way ANOVA model of minnow schooling behaviour, measured as the proportion of focal school composition during schooling. The factors are familiarity and threat. Data were arcsine transformed. * indicates an interaction term between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	2170.5	18.09	0.000
Threat	1	0	0	1.000
Familiarity * threat	1	8.8	0.07	0.788
Error	76	120.0		

Table 6. Two-way ANOVA model of minnow schooling behaviour, measured as the proportion of total time in each section. The factors are threat and fluvarium section. Data were arcsine transformed. * indicates an interaction term between factors.

Source	*df*	Mean square	*F*	*P*
Threat	1	13.5	0.03	0.855
Fluvarium section	2	11209.8	27.83	0.000
Threat * section	2	2998.2	7.44	0.001
Error	114	402.8		

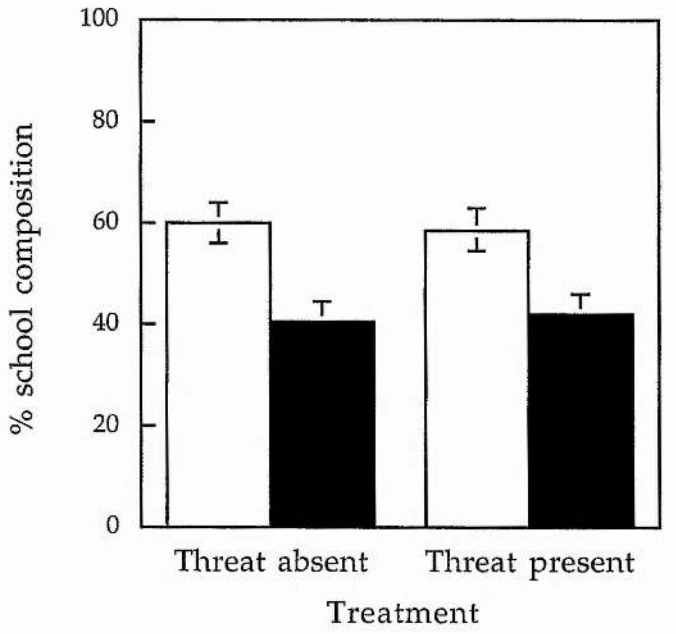

Figure 8. Composition of minnow schools in terms of familiar (❑) and unfamiliar (■) fish observed in the absence or presence of a live pike predator. Data (back transformed from arcsine data) are given as means ± 1s.e. (n=20).

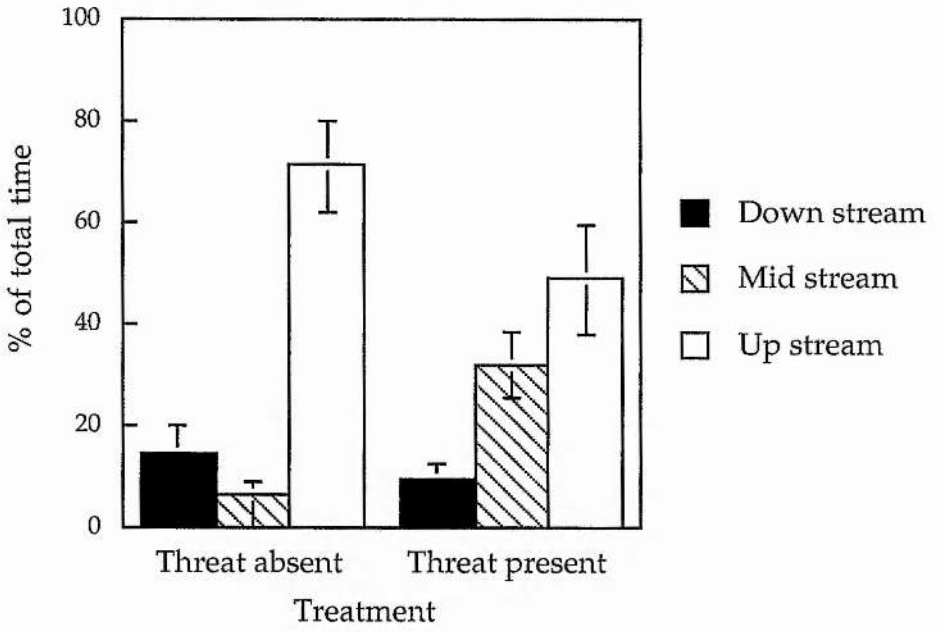

Figure 9. Distribution of minnows between upstream, midstream and downstream sections of the fluvarium channel measured as proportion of total time in each section. Data (back transformed from arcsine data) are given as means ± 1s.e. (n=20) in the absence or presence of a live pike predator.

Discussion

This experiment demonstrates that European minnows observed under near-natural conditions are able to distinguish conspecifics on the basis of prior experience and prefer to school with familiar school-mates over unfamiliar fish. There is also evidence that other fish species may form schools which are non-random assemblages of conspecifics. For example, Van Havre & FitzGerald (1988) observed the schooling preferences of wild female threespine sticklebacks allowed to choose among familiar and unfamiliar fish and found these fish prefer familiar schooling partners. Indeed, they were able to recognise their natural school-mates after 10 days separation. More recently, observations of guppy partner choice decisions have also revealed a preference for familiar conspecifics (chapter 3 and Magurran *et al.* 1994).

The minnows observed in this study were collected as naturally occurring schools, and the results of this investigation are therefore likely to be a true reflection of the schooling decisions of fish in the wild. Schools were housed separately for at least three days between capture and observation. Although it is possible that fish became more familiar with one another during this time, it is known that familiarity is acquired over a relatively long time period (about 12 days for guppies, chapter 6, if context dependent cues have not been made available). The minnows in this study might therefore be expected to have been associating with particular individuals for protracted periods in the wild in order to have recognised and demonstrated a preference for their school-mates.

The preference of minnows for familiar school-mates was not increased by predatory threat as predicted. Minnows continued to choose familiar over unfamiliar conspecifics, and school composition remained biased towards familiar fish to the same degree in the absence and presence of a pike. The pike had been fed before use in the trials, and because it was possible that the minnows recognised the pike's motivational state they may not have regarded it as a true threat. Evidence that fish are able to base decisions of anti-predator behaviour on the motivation of a potential predator is contradictory. Csányi &

Dóka (1993) found little evidence of discrimination between hungry and satiated predators by paradise fish, *Macropodus opercularis*. Similarly, sand gobies, *Pomatoschistus minutus*, do not distinguish between hungry and satiated cod, *Gadus morhua*, although hungry cod eat more gobies (Magnhagen & Forsgren 1991). However, distinction between hungry and satiated predators has been shown in the guppy (Licht 1989). Licht (1989) argues that the potential for a flexible response to differential threat which this ability affords individual guppies allows the cost of anti-predator behaviour to be reduced. An alternative scenario is that the minnows became progressively habituated to the presence of the pike during the 5 hours settling period. Since the pike had not been able to make a successful strike during this time, the minnows may no longer have regarded it as a threat. This possibility cannot be wholly discounted, although the minnows did spend less time in the upstream section containing the cage when the pike was present. It remains to be seen whether the preference of minnows for their familiar school-mates would be enhanced if they were presented with, for example, a hungry pike or a pike launching an escalated attack. The second experiment in this chapter (section 4.2) attempts to address these problems.

4.2 Do preferences for familiar minnows vary with risk of predation from a realistically painted predator model?

Introduction

The first experiment described in this chapter (section 4.1) demonstrated that minnows are able to discriminate between conspecifics, preferring to school with their natural school-mates when given the chance to do so. The majority of observed schools were found to be composed of individuals familiar to one another. The question remains, however, as to whether these familiar individuals are randomly distributed throughout the school or whether they form one or more sub-groups? In this second experiment nearest neighbour preferences were measured in order to assess partner choice decisions of individuals within schools. The effect predator threat has on partner choice decisions was investigated by using a model pike. In this way it was possible to eliminate the effect which any differences in motivational state of a live pike might have on minnow schooling behaviour between trials. Furthermore, accurate and repeatable remote manipulation of the model enabled the stalking behaviour of a live pike to be mimicked.

Methods

The experiment was undertaken during June 1996 using a fluvarium (as described above in section 4.2, in chapter 2 and plate 6) which facilitated the observation of fish behaviour in effectively natural conditions. During trials, fish were restricted to a section of one fluvarium channel measuring 4m long x 60cm wide x 35cm water depth. Water (mean temperature of 18.5°C ± 1s.e. = 0.18) flowed over a 30cm layer of gravel (mean diameter 2cm) at a mean velocity of 0.15ms^{-1} ± 1s.e. = 0.04. The fluvarium's glass roof allowed observations to be made under natural light conditions. The channel was subdivided for observation purposes into three sections (upstream, midstream and downstream) by marks on the glass walls visible to the observer. A clump of the macrophyte, *R. penicillatus*, was placed in the downstream

section and a hide concealing a model pike (details below) was located in the upstream section (figure 10).

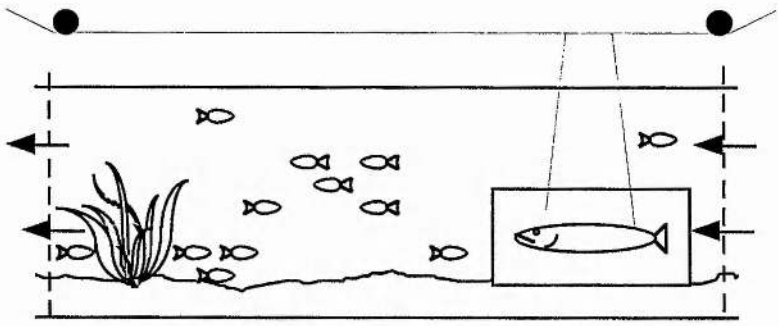

Figure 10. Schematic representation of fluvarium channel (not to scale). Arrows indicate direction of water flow and model pike in hide is positioned upstream. The model was made to advance from its hide by pulling the nylon line from which it was suspended. Other features illustrated include a clump of weed, layer of gravel and two screens which prevented the escape of the 12 minnows (6 from each of 2 schools) from the observation arena. The position and nearest neighbour preferences (familiar and unfamiliar fish) of focal minnows was observed before and after presentation of the pike model.

(a) Experiment

Schools of approximately 70 minnows were obtained from each of two sites (8 km apart) on the River Frome, Dorset by seine netting. Members of the same school were defined as 'familiar' whereas fish from different schools and different sites were defined as 'unfamiliar' to one another. These two schools were housed in visual and olfactory isolation in separate cages for 3 days before the beginning of the experiment. The fish were fed daily with trout fry pellets. Before a trial six minnows from each cage were measured (total length) and each individual given a batch mark. This consisted of a spot of Radiant® fluorescent pigment applied to each fish's caudal fin using a small paint brush. All 12 fish were then immediately placed into the fluvarium and allowed to settle for 2 hours, by which time the fish were foraging across the bottom of the gravel in small groups. In this way the schooling preferences of recently caught minnow schools could be measured. At no time during this experiment were minnows observed to be spawning.

During each trial, observations were made on a focal fish for 10 min before and after the model pike was presented. The model (total length 47.5 cm) was made of resin using the plaster cast of a dead pike. It had been painted realistically as described by Magurran *et al.* (1985) and Magurran & Girling (1986). The model was suspended from a monofilament nylon line which ran the length of the channel. During the first 10 min observation period the model remained hidden from view in the hide. After this time, the line was pulled manually so that the model was revealed to the minnows. It was made to advance from the hide through the upstream portion of the channel for a 1 min period. After this time the model remained stationary, but visible to the minnows for the remaining 9 min of the observation period. Observations of schooling behaviour and remote control of the model predator were undertaken from behind a hide to avoid disturbance to the fish. Time spent by focal fish schooling with familiar (same original school) or unfamiliar (different school) nearest leader and follower fish was measured before and after the predator model had been introduced. A schooling partner was defined for the purposes of this experiment as any fish within 3 body lengths of the focal fish. This can be regarded as a conservative definition of schooling as Pitcher (1983) considered 4 body lengths to be an appropriate measure of school membership for cyprinids. In addition, time spent by the focal fish in each of three sections of the channel, as well as proportion of the focal fish's school composed of familiar and unfamiliar conspecifics was measured every 30s. No fish was used more than once and 12 trials were completed in total.

(b) Control

To control for any schooling bias for or against different batch marks, a school of minnows was collected from the River Frome and housed in a holding cage as above. A new school of fish was used in the control trials to avoid repeated exposure and possible habituation of the minnows to the model pike. Before a trial 12 fish were removed, of which six were randomly chosen for the application of one type of batch mark, and the remaining six were marked differently. A focal fish was observed for 10 min before and after the model pike was presented in the same way as described above. 12 trials were completed in total. No fish was used more than once.

Data analysis

Preferences for familiar and unfamiliar schooling partners were expressed either as percentage of total number of fish in a school (school composition data) or as percentage of total time schooling (nearest neighbour preference data). Proportion data were used in order to account for variation in schooling tendency between schools. A two-way ANOVA was conducted to test the effects of familiarity and threat on school composition while a three-way ANOVA investigated the effects of familiarity, threat, and leader/follower preference on time schooling with nearest neighbour. All proportion data were arcsine transformed for analysis (Sokal & Rohlf 1995).

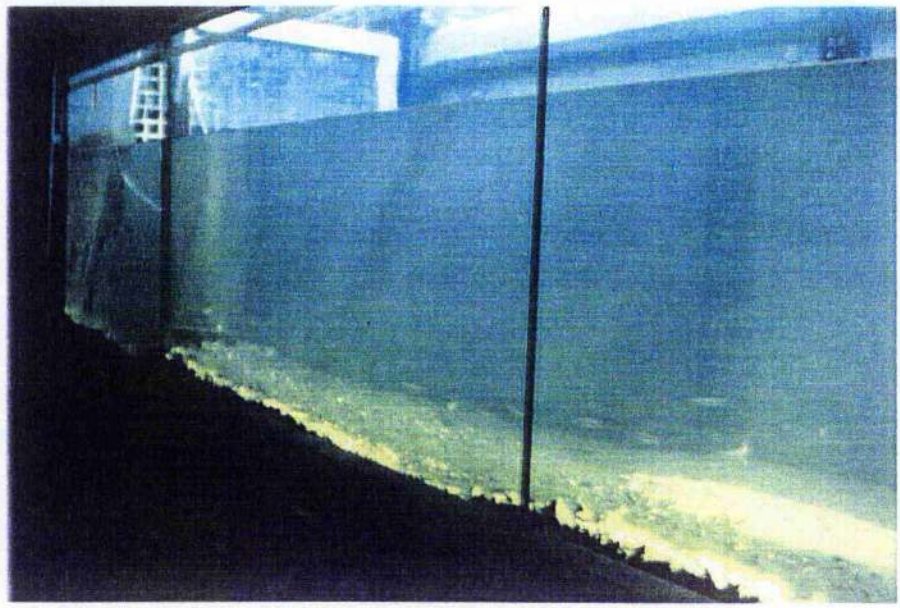

Plate 6. European minnows in the IFE fluvarium. Water flow is from the right.

Results

Minnow schools were found to be composed of significantly more familiar than unfamiliar fish ($F_{1,44} = 41.42$, $P < 0.001$, table 7), familiar individuals comprising 75% of the mean school size of 4.4 individuals (± 1s.e. = 0.4, figure 11). The total length of fish did not vary significantly between groups ($F_{1,143} = 0.03$, $P = 0.872$, mean length ± 1s.e. = 40.2 ± 0.5mm and 40.3 ± 0.5mm).

Minnows demonstrated an overall preference for familiar nearest neighbours over unfamiliar individuals ($F_{1,88} = 25.75$, $P < 0.001$, table 8, figure 12). Of the total time spent schooling, 66% was spent next to a familiar nearest neighbour. Individuals were no more likely to have a lead near neighbour than a follower near neighbour (time as percentage of total schooling: $F_{1,88} = 0.00$, $P = 1.00$, table 8). However, the interaction between familiarity and schooling partner identity (time as percentage of total schooling: $F_{1,88} = 24.90$, $P < 0.001$, table 8, figure 12) suggests that preference for familiar individuals is greater in the case of lead fish. All other interaction effects were not significant (table 8).

The presence of a model pike had no effect on time spent by focal fish schooling with nearest leader or follower neighbour (time as percentage of total schooling: $F_{1,88} < 0.001$, $P = 1.00$), nor on overall school composition ($F_{1,44} < 0.001$, $P = 1.00$). Threat had no effect on mean school size (one-way ANOVA, $F_{1,23} = 0.12$, $P = 0.74$). However, the minnows inspected the model pike at a rate of 4.2 inspections every 10 min, demonstrating that it was identified as a potential threat.

The control experiment demonstrated that there was no inherent schooling preference for, or avoidance of, either batch mark ($F_{1,44} = 1.49$, $P = 0.23$), and that nearest neighbours were no more likely to have one batch mark than the other ($F_{2,132} = 0.93$, $P = 0.935$). Interaction effects were not significant. The total length of fish did not vary significantly between groups ($F_{1,143} = 0.65$, $P = 0.420$, means ± 1s.e. = 42.2 ± 0.5mm and 42.8 ± 0.5mm). The presence of a pike model had no effect on school composition ($F_{1,44} = 0.00$, $P = 1.00$). Mean school size did not vary with presence or absence of the predator model ($F_{1,23} = 2.24$, $P = 0.149$).

Table 7. Two-way ANOVA model of minnow schooling behaviour, measured as the proportion of focal school composition during schooling. The factors are familiarity and threat. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	10758.2	41.42	0.000
Threat	1	0	0	1.000
Familiarity * threat	1	21.3	0.08	0.776
Error	44	259.7		

Table 8. Three-way ANOVA model of minnow schooling behaviour, measured as the percentage of time spent with nearest neighbour fish during schooling. The factors are familiarity, threat and leader/follower preference. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	11119.3	25.75	0.000
Threat	1	0	0	1.000
Leader/follower	1	0	0	1.000
Familiarity * threat	1	15.0	0.03	0.853
Familiarity * lead/foll.	1	10751.0	24.90	0.000
Threat * lead/foll.	1	337.5	0.78	0.379
Familiarity * threat * lead/foll.	1	479.6	1.11	0.295
Error	88	431.8		

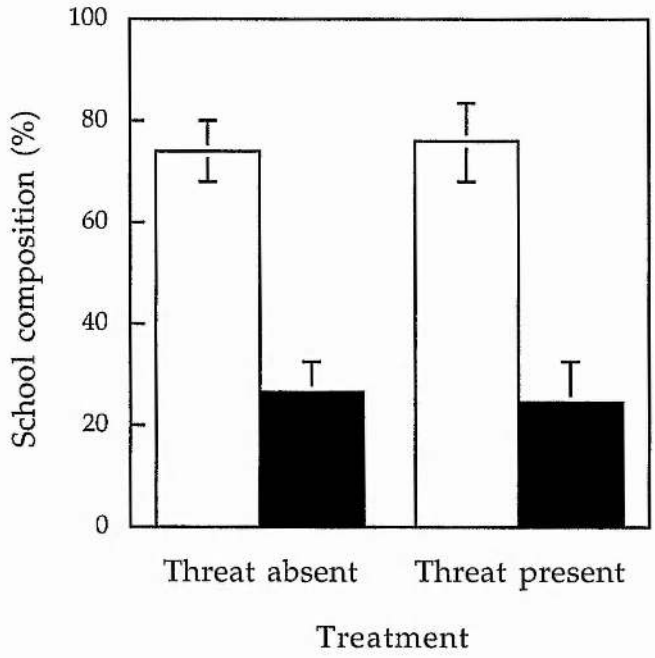

Figure 11. Composition of minnow schools in terms of familiar (❑) and unfamiliar (■) fish observed before and after introduction of a model predator. Data (back transformed from arcsine data) are given as means ± 1s.e. (n=12).

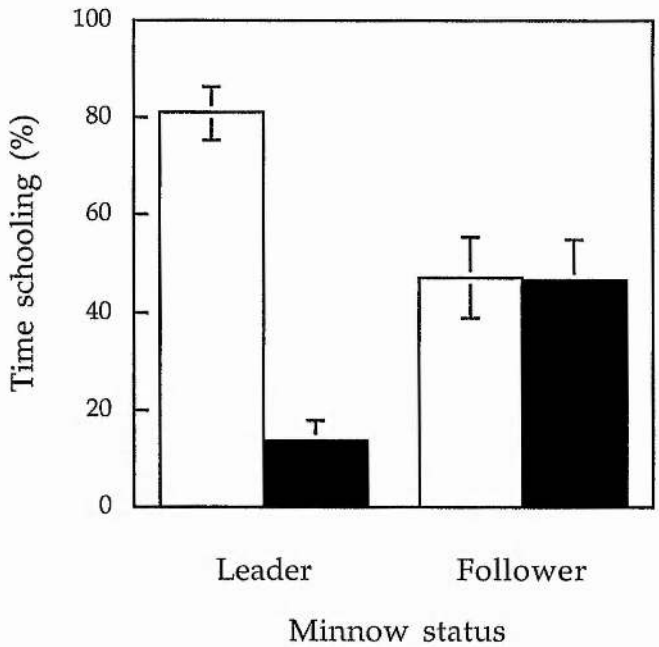

Figure 12. Nearest neighbour preferences of minnows in terms of the percentage of total time schooling (back transformed from arcsine data) spent with familiar (❑) and unfamiliar (■) leader and follower fish. Data are given as means ± 1s.e. (n=12).

Discussion

The results of this experiment confirm the conclusions reached by experiment 1 (section 4.1). It has been demonstrated that European minnows observed under near-natural conditions are able to distinguish conspecifics on the basis of prior experience and prefer to school with familiar school-mates over unfamiliar fish. Not only was the greater proportion of a school composed of familiar fish (75%) but, of total time spent schooling, minnows spent more time next to familiar than unfamiliar nearest neighbours. Intriguingly, the preference of minnows for familiar conspecifics was only documented for lead fish. However, it is possible that this can be attributed to observer difficulties in viewing the positions and identity of both lead and follower fish in a moving three-dimensional school, precedence being given to the focal fish's leader. Nonetheless, it is the schooling decisions of the focal fish which can be regarded as the most important aspect of this work, these being most clearly demonstrated in the choice of lead fish.

It could be argued that minnows were preferentially associating with familiar conspecifics on the basis of similarity in appearance. Fish are known to prefer school-mates of similar size (Ranta *et al.* 1992b; Krause *et al.* 1996b) and choose partners of particular colour (e.g. Houde 1987). If this were the case for River Frome minnows the schooling preferences described in this experiment might be due to familiar individuals sharing batch marks. The control experiment discounts this possibility. Minnows do not prefer or avoid conspecifics marked with either of the colours used.

Although this study clearly documents the preference by minnows for familiar school-mates, contrary to expectation this preference was not increased by predatory threat. Minnows continued to choose familiar over unfamiliar near neighbours and school composition remained biased towards familiar fish to the same degree before and after presentation of the model pike in the same way as recorded in experiment 1 (section 4.1) and chapter 3. Because the materials and methodology used in this study closely resembled that of

Alan & Pitcher (1986) and Magurran *et al.* (1985), there is no reason to suppose this result is an artefact of the experimental conditions. Models have been used successfully in the past to simulate predatory attack (Magurran *et al.* 1985; Allan & Pitcher 1986; Magurran & Girling 1986) and since minnows in this experiment were not exposed to the model more than once, there was no opportunity for habituation. It does not seem, therefore, that use of a live piscivore in the way described in experiment 1 (section 4.1) achieves a greater degree of realism than can be gained by using a model. Furthermore, the use of models in behavioural studies avoids the ethical problems of placing fish under true threat of predation (Huntingford 1984).

Allan & Pitcher (1986) observed schools of dace, gudgeon, *Gobio gobio*, and European minnows during simulated predator attack and measured a decrease in the number of mixed species schools while the number of single species groups increased. Mixed schools of coral reef fish are also known to segregate when under threat (Wolf 1985), as do other animals, especially birds (Powell 1974; Bertram 1978; Caraco 1980). Individuals abandon a mixed group quickly if the number of conspecific members is low, but continue to school with heterospecifics despite being threatened if enough conspecifics are present (Wolf 1985). By making partner choice decisions on the basis of species identity when threatened, the minnows observed by Allan & Pitcher (1986) presumably gained anti-predator advantages. It is possible that these anti-predator advantages also could be acquired by fish choosing to school with particular types of conspecific. Chivers *et al.* (1995) demonstrated that schools of familiar fathead minnows exhibited better anti-predator behaviour when subjected to chemical stimuli from pike, and to a pike model, than schools of unfamiliar fish. Schools of minnows familiar to one another demonstrated tighter school cohesion and increased number of predator inspections (Chivers *et al.* 1995). This suggests that fish in the wild should gain significant advantages by joining a familiar school of conspecifics in the event of a predatory attack.

4.3 Further work

In future it would be interesting to determine whether populations of fish exposed to different levels of predation pressure express their preference for familiar school-mates differently, or indeed if schooling decisions of individual fish vary with the level of predation risk. River Frome minnows are exposed to predation pressure from many species of fish (see chapter 2), the most notable of which is the pike, which occurs in densities of approximately one every 10m (Mann 1980). Mann (1982) found minnows to comprise up to 50% of the diet of young pike in the River Stour, southern England. Fish from highly predated populations such as River Frome minnows may routinely prefer to school with familiar associates.

Because familiarity develops gradually (chapter 6) and fish may have to associate with one another for relatively long time periods in the wild in order to become familiar, the choice of schooling partner may have important evolutionary consequences for fish populations, especially if current research reveals a role for kinship in schooling decisions (chapter 8). So far, however, there is little evidence to support the idea that schools are composed of related individuals (Avise & Shapiro 1986; Naish *et al.* 1993, and see chapter 8). There have been few other attempts to investigate the schooling preferences of wild fish and evidence of school fidelity in the wild is sparse and equivocal at present. Helfman (1984) provides an exception in having observed the behaviour of 102 individually identifiable yellow perch, *Perca flavescens*, in a population in Cazenovia Lake, U.S.A., finding little evidence for associations among particular individuals. Work is required to provide information on the home ranges as well as the seasonal and daily movements of fish in the wild before individual partner choice decisions can be fully understood.

4.4 Summary

In the near-natural conditions of a fluvarium in the River Frome, U.K., European minnows displayed schooling preferences for familiar conspecifics during two investigations. Schools were composed of 59% and 75% familiar fish. The minnows in this river co-occur with many piscivores, the most notable being the pike. The preference for familiar school-mates was not increased by the threat of predatory attack from a model pike or a live (but caged) pike despite the anti-predator benefits afforded to individual members of schools of familiar fish. It may be that River Frome minnows routinely school with familiar conspecifics.

CHAPTER 5

Is there a sex difference in preferences for familiar school-mates[1]?

5.1 Introduction

The asymmetrical mating costs experienced by males and females (Trivers 1972; Parker 1983) have far reaching consequences. It is usually females which invest most time and energy in their offspring (Bateman 1948) and tend to be the choosy sex. Males have a much higher potential reproductive output. However to realise this, they must be attractive to many partners, or be able to out-compete male rivals and undermine female choice (Clutton-Brock & Parker 1995). In the case of fish, female reproductive success is limited by body size as the number of eggs produced increases with increasing body size. Male reproductive success on the other hand is limited only by the number of matings achieved. These differences lead the two sexes to follow separate agendas for much of their lives, even when engaged in activities, such as anti-predator behaviour (Sih 1994), not directly related to reproduction.

Schooling behaviour in fish functions primarily as a defence against predators (Pitcher & Parrish 1993) - reviewed in chapter 1. Schools were once portrayed as egalitarian groupings in which individuals co-operated to produce co-ordinated anti-predator manoeuvres for the common good (Breder 1954; Radakov 1973). Evolutionary biologists soon realised that individual members continually re-appraise the costs and benefits of belonging to a school (chapter 1) and will join or leave when it is rewarding to do so (see review by Pitcher & Parrish 1993). It has also become clear that individual decisions determine not only school membership but also school composition and structure. Wild schools are sorted by species and body size (Krause *et al.* 1996a) and individuals attempt to adopt

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[1]Data from part of this study has been submitted for consideration for publication as Griffiths, S. W. & Magurran, A. E. (submitted) Sex and schooling behaviour. Anim. Behav.

positions within schools that provide foraging (Krause *et al.* 1992) and anti-predator advantages (Hamilton 1971; Krause 1993a). Krause (1992) demonstrated that juvenile roach, *Rutilus rutilus*, have positional preferences within a school which lead to hungry individuals occupying front positions where they gain foraging advantages. Furthermore, it is known that centrally located fish are at a lower risk of predation than those at the periphery of the group (chapter 1). Individuals in the so called 'selfish herd' seek cover behind other individuals by trying to obtain central positions (Hamilton 1971).

An individual's fitness may be further enhanced if they preferentially school with certain individuals and previous experience, usually termed familiarity, appears to play an important role in choice of schooling partner in threespine sticklebacks, *Gasterosteus aculeatus* (Van Havre & FitzGerald 1988), fathead minnows, *Pimephales promelas*, (Brown & Smith 1994), guppies, *Poecilia reticulata*, (Magurran *et al.* 1994; Warburton & Lees 1996; Griffiths & Magurran 1997b), and bluegill sunfish, *Lepomis macrochirus*, (Brown & Colgan 1986) - see table 2 and chapter 1. Anti-predator behaviour of fathead minnows is more effective if a school consists of familiar individuals (Chivers *et al.* 1995).

Does gender also mediate schooling decisions? It has already been shown that the sexes may differ in their schooling tendency. For example, female guppies in Trinidad spend more time schooling and invest greater effort in anti-predator behaviour than males (Magurran & Seghers 1994c). Since fecundity is related to longevity it is clearly in a female's best interests to avoid predation. A female guppy will produce a brood at approximately monthly intervals for most of her life; sperm storage ensures that she can fertilise new embryos even if she has been unable to remate (Constanz 1989). Males, by contrast, devote much of their time to courtship activities (Magurran & Seghers 1994c) and constantly follow females in an attempt to secure more matings, even at the cost of an increased predation risk (Godin & Dugatkin 1996). The different trade-offs that males and females make between reproductive behaviour and predator avoidance mean that although both sexes should gain anti-predator advantages by schooling with familiar individuals (Chivers *et al.* 1995), it is females that stand to benefit most by exhibiting this behaviour in the wild. Because the schooling

behaviour of wild guppies in Trinidad has become a classic example of evolution in action (Magurran 1996) and a large body of information describing the behavioural differences between the sexes already exists, guppies make an ideal species for this investigation. This study therefore tests the hypothesis that female guppies associate preferentially with other (familiar) females from their natural wild school while males do not display such a preference.

5.2 Methods

The guppies used in this study were obtained from the Tacarigua River in Trinidad's Caroni drainage system (see chapter 2) during December 1996. This is a high predation system (Magurran & Seghers 1994b) where guppies co-occur with a range of predators including the pike cichlid, *Crenicichla alta,* and the blue acara, *Aequidens pulcher*. The guppies in this river have a high schooling tendency (Magurran & Seghers 1994b).

Schools of guppies which comprised males, females and juveniles, were gently collected in their entirety from the shallow margins of the Tacarigua River with a one-man seine net. Two schools (obtained from a distance of at least 10m apart) were captured during one field trip and returned to the laboratory. Five trips were made in total. The schools were held overnight in separate aquaria (45 x 32 x 32cm deep) in order to acclimate before observations were made the following day. Each tank contained an air stone and water temperature was 26.8 ^{o}C ± s.d. = 0.4. The fish were fed with Tetramin™ commercial fish food at night and in the morning before trials began. Fish collected in the same school were designated 'familiar' fish whereas fish in different schools were classified as 'unfamiliar'.

Individual male and female guppies were tested for their schooling preferences for familiar and unfamiliar same-sex fish. Guppies were only tested with stimulus fish of their own sex because schooling behaviour might otherwise have been confounded with courtship activity. Schooling tendency was measured using a procedure closely resembling that adopted by Magurran *et al.* (1994) after Keenleyside (1955). Two clear plastic 1l bottles (22cm high) were

positioned 7cm from either end of a test tank (60 x 30 x 30cm filled with water to 18cm). The bottles were punctured to allow chemosensory as well as visual communication. Before a trial, four equally sized stimulus fish (of the same-sex as the test fish) from each of the two schools were placed in two separate bottles. In this way the test guppy had the opportunity to school with either familiar (from its own school) or unfamiliar (from another school) fish, or to remain solitary. After 30 min settling time, the test fish was released into the centre of the aquarium.

Schooling behaviour was measured by recording the length of time the test fish spent within 7cm (3-4 body lengths) of each bottle. Trials lasted 15 minutes. 4 females and 4 males, randomly chosen from each school were tested. 10 wild schools were studied in total and in each case 4 randomly selected males and females were tested. The position of the bottles was alternated, and no fish was tested more than once. Stimulus fish were never used as test fish, nor *vice versa*. All fish were weighed and measured at the end of the trial.

Plate 7. The Upper Tacarigua River, Caura Valley, northern mountain range, Trinidad during December 1996.

5.3 Results

A three-way ANOVA investigated the effects of sex, familiarity and group (school) on time spent schooling data. None of these factors significantly affected the time spent schooling by guppies (table 9). However, a significant interaction between familiarity and sex (table 9) indicated that females differ from males in their schooling preferences. Inspection of the data for each sex demonstrates that females prefer to school with familiar school-mates (one-way ANOVA $F_{1,79} = 6.01$, $P < 0.02$, figure 13) while males do not distinguish between same-school (familiar) or different school (unfamiliar) conspecifics (one-way ANOVA $F_{1,79} = 0.25$, $P = 0.617$).

The number of individuals within the wild schools ranged from 21-54: on average schools contained 19.7 females, 15.8 males and 2.7 juveniles. The mean wet weight (g) and total length (mm) of the fish was: females 0.12g (± s.d. = 0.08) & 21.58mm (± s.d. = 4.48), males 0.08g (± s.d. = 0.03) & 19.82mm (± s.d. = 2.34) and juveniles 0.02g (± s.d. = 0.01), & 10.75mm (± s.d. = 2.55). The total length of stimulus fish did not vary amongst schools (one-way ANOVA, females: $F_{9,40} = 1.76$, $P = 0.117$ and males: $F_{9,40} = 1.70$, $P = 0.133$), indicating that schooling preferences were not based on fish size.

Table 9. Three-way ANOVA of guppy schooling behaviour, measured as total time spent schooling with stimulus fish in choice tests. The factors are familiarity, sex and group. * indicates an interaction between factors.

Source	df	Mean square	F	P
Familiarity	1	50446	1.74	0.190
School (group)	9	9392	0.32	0.966
Sex	1	14803	0.51	0.477
Familiarity * group	9	18736	0.65	0.757
Familiarity * sex	1	147198	5.07	0.026
Group * sex	9	5435	0.19	0.995
Familiarity * group * sex	9	20831	0.72	0.692
Error	120	29045		

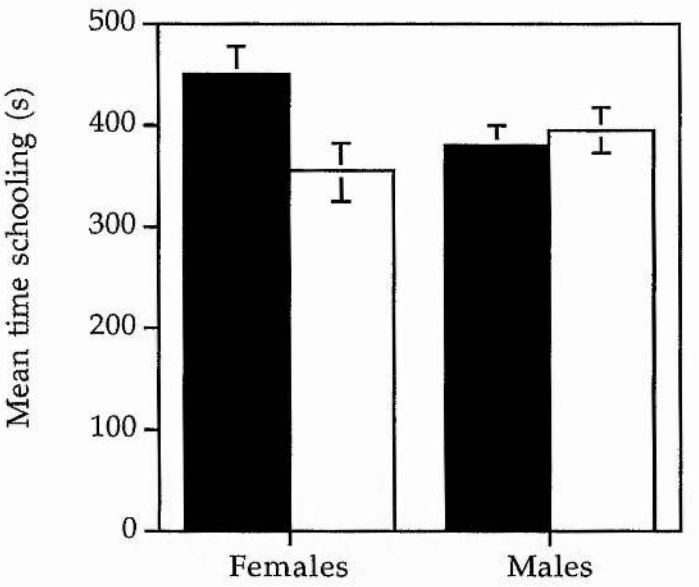

Figure 13. Sex differences in the preference for natural school-mates by wild Tacarigua guppies in a bottle choice test. Solid bars indicate preferences for schooling with same-sex individuals from the natural school while open bars represent preferences for guppies from an unfamiliar school. Mean time schooling with stimulus fish (± 1s.e.) is given. 40 different fish of each sex were tested.

5.4 Discussion

This study shows that wild female guppies prefer to school with familiar conspecifics whereas males do not make this distinction. Individual females spent, on average, 57% of their schooling time with females from the school they belonged to in the wild. The equivalent figure for males was 49%.

It is known that familiarity takes time to develop in the absence of condition dependent cues. Chapter 6 describes a laboratory study, in which it was only after 12 days that female guppies began to show a schooling preference for fish from their home tank (Griffiths & Magurran 1997a). This implies that the females in this study must have been together for protracted periods in the wild in order to recognise and preferentially associate with their school-mates.

The results suggest that wild guppy schools are not random assemblages of conspecifics. Instead, the picture that emerges is one in which females form the core of natural schools. These females can recognise one another, and choose to remain together, even though a river, unlike an aquarium, offers many opportunities for dispersal. Why are partner preferences for familiar fish only displayed by females? The advantages to individuals of discrimination on the basis of familiarity have only begun to be elucidated in recent years. However, schools of familiar fathead minnows are known to display better anti-predator behaviours than schools of unfamiliar individuals (Chivers *et al.* 1995), while European minnows, *Phoxinus phoxinus*, choose the poorest competitors in future feeding bouts so as to gain foraging advantages (Dugatkin & Wilson 1992; Metcalfe & Thomson 1995). Males appear to trade-off these advantages against increased mobility which will enable them to move opportunistically amongst schools in search of mating opportunities.

Wild guppy schools consist of groups of familiar females. Could these females also be kin? Fish species are likely to vary enormously in their propensity to form schools of related individuals. For instance, it seems improbable that relatives in species with broadcast spawning or

planktonic larvae will become reunited to form adult schools. Nonetheless there are fish whose reproductive biology could favour the prolonged association of kin and one of the best examples of these is the guppy, since it is an ovoviviparous species in which juveniles school from birth. A recent study by Warburton & Lees (1996) provides evidence of kin discrimination in a domestic guppy strain and chapter 8 investigates the possibility of kin recognition in a wild guppy population. Multilocus fingerprinting of wild guppy schools has also revealed that school members are more closely related than the average for the population (Magurran *et al.* 1995). However, the level of relatedness within schools was low, in the range of 4th order relatives (Lynch 1988) - a finding that could possibly be attributed to the fact that the schools were analysed without regard to sex. On the basis of this experiment it is predicted that female members of guppy schools are much more likely to be kin than males (see chapter 9).

Examples of sex biased natal dispersal, movement of animals from place of birth to place of reproduction, have been documented in a diverse range of animals including mammals, birds and insects (Greenwood 1980; Dobson 1982). For example, male African wild dogs, *Lycaon pictus*, disperse in larger groups as well as further than females, the more philopatric sex (McNutt 1996). Philopatry is also female-biased in the case of the harbour porpoise, *Phocoena phocoena*, (Walton 1997), side-blotched lizard, *Uta stansburiana*, (Doughty *et al.* 1994) and bushtail possum, *Trichosurus vulpecula*, (Clout & Efford 1984). The suggested benefits to males and females of sex-biased natal dispersal are reviewed by (Moore (1993) who lists the two main hypotheses as the avoidance of inbreeding and the reduction of intrasexual competition for resources. For female guppies, the costs of potential inbreeding and resource depletion may be outweighed by the benefits of co-operative partnerships. In any case male mobility minimises the likelihood of inbreeding.

There are two potential evolutionary consequences of this sex difference in behaviour. First, co-operative behaviour, such as that displayed during predator inspection (Milinski *et al.* 1997) should be more prevalent amongst females than amongst males. Dugatkin (1995) notes that knowledge of an individual's response during previous encounters with a predator is an important criterion when selecting

partners for inspection. Females that associate for protracted periods in the wild should be well informed about their school-mates' behaviour and 'trustworthiness'. Dugatkin (1991a) however found that both male and female guppies from a high-risk population displayed all the behavioural characteristics associated with the Tit-for-Tat strategy.

Second, the separate agendas of the two sexes may have implications for gene flow and population differentiation. Many attributes of female behaviour, including the female associations described here, should hasten the spatial and genetic sub-structuring of guppy metapopulations (Hastings & Harrison 1994; Harrison & Hastings 1996). Indeed, guppy populations are a classic example of natural selection in action and differ in a wide range of traits including life-history, morphology and behaviour (Endler 1995). Nonetheless, although many of the factors that should facilitate rapid evolution are present, Trinidadian guppy populations are not speciating (Endler 1995). Male mobility and mating behaviour may be the key to understanding why gene flow in this species (Haskins *et al.* 1961; Slatkin 1985) is sufficient to prevent populations becoming reproductively isolated (Magurran 1996).

5.5 Summary

This study tests the hypothesis that sexual asymmetry in mating costs affects partner choice decisions during schooling in fish. It is shown that female guppies from the Tacarigua River, Trinidad, associate preferentially with other (familiar) females from their natural wild school, while males do not display such a preference. This implies that wild guppy schools are not random assemblages of conspecifics. Females form the core of natural schools while males seem to trade-off the potential advantages of schooling with familiar conspecifics against increased mobility in search of mating opportunities. The implications of these findings are discussed in relation to co-operative behaviour, gene flow and population differentiation.

CHAPTER 6

Familiarity in schooling fish: how long does it take to acquire?[1]

The preference of fish for familiar school-mates is now a well documented phenomenon (reviewed in chapter 1), and although benefits are available to fish which demonstrate this discriminatory ability (Chivers *et al.* 1995; Metcalfe & Thomson 1995), little is known of how recognition is achieved. Discrimination between conspecifics is especially challenging since it must be reconciled with similarity in appearance among school members due to the oddity effect (Landeau & Terborgh 1986), where odd-looking individuals are at greater risk of predatory attack. In cases where morphological differences exist, fish can easily distinguish school-mates (e.g. Houde 1987). Where individuals closely resemble one another, however, the question remains as to how long it takes for fish to be able to discriminate between conspecifics on the basis of familiarity alone.

6.1 Introduction

The discriminatory abilities of fish have inspired an increasing amount of interest during recent years. Partner choice preferences have been documented in the contexts of foraging behaviour e.g. threespine sticklebacks, *Gasterosteus aculeatus* (Ranta & Lindström 1990), striped parrotfish, *Scarus iserti* (Clifton 1991) and bluegill sunfish, *Lepomis macrochirus* (Dugatkin & Wilson 1992); anti-predator behaviour e.g. threespine sticklebacks (Milinski *et al.* 1990a) and guppies, *Poecilia reticulata* (Dugatkin & Alfieri 1991a), as well as extensively in the field of mate choice e.g. guppies (Dugatkin & Sargent 1994) and parasite avoidance e.g. threespine sticklebacks (Dugatkin *et al.* 1994) - reviewed in Chapter 1. Fish choose to associate with particular conspecifics and this ability to discriminate between potential school-mates plays an

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[1] Data from part of this study has been published as Griffiths, S. W. & Magurran, A. E. (1997) Familiarity in schooling fish: how long does it take to acquire? Anim. Behav. 53, 945-949.

important role in individual decision making (Dugatkin & Sih 1995). Threespine sticklebacks, (Milinski *et al.* 1990a; 1990b) and guppies (Dugatkin & Alfieri 1991a) remember the outcome of past encounters when selecting partners for predator inspection, choosing the most trustworthy individuals.

Schooling behaviour is a highly effective strategy against predators (Magurran 1990a) and it has been shown that larger schools are not only more vigilant (Magurran *et al.* 1985), but also less vulnerable to attack by pike, *Esox lucius*, and other predators including squid, *Loligo vulgaris*, cuttlefish, *Sepia officinalis*, and perch, *Perca fluviatilis* (Neill & Cullen 1974). In the case of pike, Neill & Cullen (1974) demonstrated that an increase in school size from one to six prey fish (bleak, *Alburnus alburnus* and dace, *Leuciscus leuciscus*) reduced capture rate by 26% suggesting a decreased per capita predation risk to fish schooling in large numbers. More recently Krause & Godin (1995) investigated risk of predation to guppies schooling in groups of 2, 5, 10 or 16 individuals at different water temperatures (where cold water decreased fish activity levels). In their laboratory experiment the blue acara cichlid, *Aequidens pulcher*, preferred to attack schools displaying high activity levels, irrespective of school size, thus demonstrating the importance of visual conspicuousness to predator attack. Although it is recognised that individual fish which differ in appearance or behaviour may be at greater risk than their school-mates as a consequence of the oddity effect (Landeau & Terborgh 1986), scant attention has been paid to how school membership influences the effectiveness of schooling as an anti-predator device. For instance, there has been little consideration of the way in which individual recognition might enhance the anti-predator benefits of schooling, despite extensive studies of predator inspection behaviour which have demonstrated, for example, that guppies and sticklebacks prefer the most co-operative of inspection partners (Milinski *et al.* 1990b, respectively; Dugatkin & Alfieri 1991b). However, a recent laboratory investigation of fathead minnows, *Pimephales promelas*, revealed a difference in anti-predator responses between schools of fish collected from the wild as naturally occurring schools (and thus being composed of familiar individuals) and groups of fish composed of individuals from 4 different naturally occurring schools, and thus being composed of unfamiliar individuals (Chivers *et*

al. 1995). Chivers and his colleagues found 'familiar' schools to be more cohesive than 'unfamiliar' schools, more frequently adopting behaviours, such as predator inspection, that bestow greater protection on school members (Chivers *et al.* 1995). These results, along with other studies that have demonstrated schooling preferences for familiar fish (table 2, Van Havre & FitzGerald 1988; Magurran *et al.* 1994; Chivers *et al.* 1995) imply that individual recognition is a neglected aspect of schooling dynamics.

Individual recognition may be beneficial but how is it achieved? Morphological differences between individuals allow discrimination to be made on the basis of visual cues. For example Houde, (1987) noted a preference by female guppies for males displaying the brightest orange colouration. However, the oddity effect selects against individual variation in appearance so that, for example, colour-dyed fish are more likely to be preyed upon when returned to their natural school (Hobson 1968; Landeau & Terborgh 1986). In the wild, fish are found to be segregated according to similarity in phenotype (Krause *et al.* 1996a). Indeed, one of the most striking features of natural schools of fish is the degree to which the school members resemble one another. Thus the very factor that confers protection, similarity in appearance, is also one that makes individual recognition more challenging. While the ability of fish to distinguish between conspecifics on the basis of morphological differences may be demonstrated with apparent ease (Ranta *et al.* 1992b), recognition based on familiarity may be slower to develop.

In the case of the threespine sticklebacks, Ranta *et al.* (1992b) have shown that individuals in a laboratory experiment choose schooling partners according to which of the potential school-mates have body sizes best matching their own. Observations of wild sticklebacks presented in the same study (Ranta *et al.* 1992b) lend further support to the size assortative hypothesis. Traps were set in the wild, each trap containing either small sticklebacks (~4cm) or large sticklebacks (~5.5cm). Schools of sticklebacks subsequently observed near the traps containing the small-sized conspecifics were themselves small in 12 of 18 occasions, and large in 17 of the 20 occasions when the trapped fish were also large (Ranta *et al.* 1992b).

Previous investigations of the role of familiarity in decision-making have examined groups of fish that were kept together for considerable periods of time (table 2). Magurran *et al.* (1994) looked at schooling preferences for familiar fish in groups of (<15) guppies that had been together for two months. Brown & Smith (1994) and Chivers *et al.* (1995) collected naturally occurring schools of fathead minnows from the wild and then maintained the schools in separate aquaria until they were ready to be tested. This period of time was at least 4 weeks in the case of Brown & Smith (1994). Metcalfe & Thomson, (1995) examined the schooling preferences of European minnows, *Phoxinus phoxinus*, kept in groups of seven for between 12 and 20 days (N. B. Metcalfe, pers. comm.) while Van Havre & FitzGerald (1988) showed that threespine sticklebacks preferred to school with conspecifics from their natural school after having been collected from the wild and housed together for two weeks. Moreover, bluegill sunfish which had been in groups of six for >3 months preferred to associate with familiar individuals (Dugatkin & Wilson 1992). How long does it take for fish to begin to treat 'familiar' individuals as preferential schooling partners? To answer this question the schooling preferences of female guppies were tested in the laboratory.

Female guppies are cryptic and uniform in appearance, unlike their male counterparts which are brightly coloured and so polymorphic that no two individuals resemble one another (Magurran *et al.* 1995). Female guppies also have a higher schooling tendency than males and are more likely to adopt anti-predator tactics in threatening situations (Magurran & Seghers 1994c). The population of guppies used in this experiment, the Lower Tacarigua, is derived from a site where there are many predators (see chapter 2). As might be expected, females from this population school strongly (Magurran & Seghers 1994b) and have the potential to benefit from the 'familiarity' benefits that Chivers *et al.* (1995) identified. Over what time frame can such familiarity be established?

Plate 8. One female (bottom right) and two male Trinidadian guppies

6.2 Methods

The individuals used in this experiment were normally held as large breeding stocks in the laboratory, and were descendants of guppies collected from the wild in Trinidad 18 months previously. Thirty-six females of as similar a size as possible (mean total length = 31.8 ± 0.58mm) were removed from three stock tanks and placed in an aquarium together for four days to allow complete mixing before separating them into six groups (figure 14). Each group of six fish was allocated its own aquarium (45 x 32 x 32 cm deep) so that the groups were isolated visually and olfactorily from one another. Each tank contained a water filter, air stone and clump of Java moss, *Vesicularis dubyana*. The fish were fed daily with Tetramin™ and kept at 25°C on a 12 h light regime. Fish which shared a tank were designated 'familiar' fish whereas fish in different tanks were known as 'unfamiliar' fish.

There were two parts to the experiment. First, over a one month period individual guppies were repeatedly tested for their schooling preferences for familiar and unfamiliar fish. Second, to investigate the possible effect of repeated exposure to the experimental procedure on the acquisition of familiarity, a further 36 females were selected (mean total length = 32.4 ± 1s.e. 0.62mm). In this control experiment individuals were only given the choice between schooling with familiar and unfamiliar fish once, that is 12 days after they had been separated into 6 groups.

Individual schooling tendency was measured using a similar procedure to that adopted by Magurran *et al.* (1994) after Keenleyside (1955). Two clear plastic 1l bottles (22 cm high) were positioned 6 cm from either end of a test tank (90 x 32 x 32 cm filled with water to 20 cm). The bottles were punctured to allow visual and chemosensory communication. Before a trial, four females from each of two randomly chosen groups were placed in two separate bottles. It was intended that the test guppy had the opportunity to school with either the familiar (from its own tank) and unfamiliar fish (from another tank) or to remain solitary (figure 14). After 15 min settling time, the test fish was released into the centre of the test aquarium. The trials were conducted

on days 1, 2, 4, 8, 12, 15, 19, 22 and 26, as well as on day 12 of the control experiment.

Schooling behaviour was measured by recording the length of time the test female spent within 10 cm of each bottle. Trials lasted 15 min. Two fish per group were tested each day. The order of the trials was randomised, and no fish was tested more than once each day. The position of the bottles was also randomised. The total length of the fish in each group was measured at the end of the experiment in order to confirm that fish size did not vary significantly between tanks.

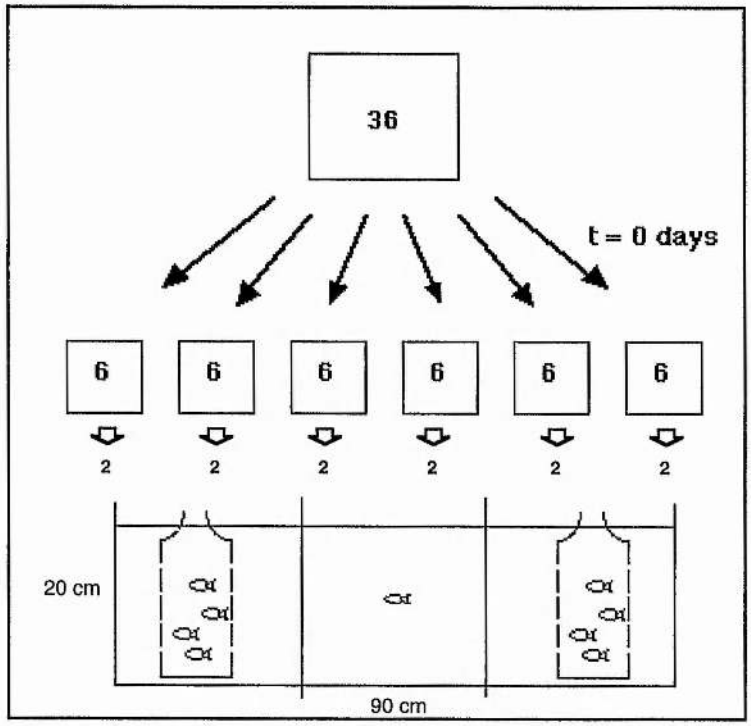

Figure 14. A schematic representation of the experimental procedure. 36 female guppies were separated into 6 groups of 6 fish each at the beginning of the experiment (day 0). Two fish from each group were subsequently given the opportunity (over a period of 1 month) to school with either familiar (same group) and unfamiliar fish (different group) or to remain solitary. Stimulus fish were held in bottles which were transparent and punctured allowing maximum opportunity for visual and chemosensory communication. The experimental tank was divided into 3 sections with marks drawn on the glass.

6.3 Results

All proportion data were arcsine transformed (Sokal & Rohlf 1995) before analysis. Since there was no significant difference in the percentage of time that the two test females from each group spent schooling (one-way ANOVA $F_{1,214} = 0.32$, $P = 0.57$), data from both females were included in the analyses of schooling preference. The statistical analyses used was a repeated measures ANOVA in which day of testing was nested within group. Overall, female guppies were shown to prefer to school with familiar rather than unfamiliar conspecifics, ($F_{1,108} = 9.43$, $P < 0.005$, table 10). The day of testing did not affect the proportion of time spent schooling ($F_{48,108} = 0.15$, $P = 0.100$). There was a significant interaction between familiarity and day ($F_{48,108} = 1.86$, $P < 0.005$) indicating that a preference for familiar fish varied with time (figure 15a; table 10). The significant interaction between group and familiarity ($F_{5,108} = 2.49$, $P < 0.05$) was due to the different rates at which groups developed preferences for familiar fish. This, however, did not change the overall preference for schooling with familiar conspecifics.

Day 12 was chosen as a suitable time for the control test as this seemed to be the critical point in the development of familiarity. After this time female guppies maintained a preference for familiar rather than unfamiliar school-mates (figure 15a). Once again, there was no significant difference in the proportion of time that the two test fish in a group spent schooling (one-way ANOVA $F_{1,22} < 0.01$, $P = 0.995$) and the results were pooled for the analysis of schooling preference. It was found that at day 12 of the control, female guppies with no previous experience of the experimental set-up or procedure demonstrated a preference for schooling with familiar school-mates (one-way ANOVA $F_{1,12} = 5.10$, $P < 0.05$, figure 15b). Interaction effects were not significant. The mean percentage time spent schooling with familiar fish in this control (56.4 %) was comparable to the value obtained on day 12 in the initial test (51.2 %). This shows that the acquisition of familiarity by day 12 was not a consequence of repeated exposure to the experimental procedure.

The total length of the guppies did not vary significantly amongst groups in either the first experiment ($F_{5,31}$ = 1.26, P = 0.307) nor the control experiment ($F_{5,31}$ = 0.26, P = 0.932) demonstrating that individuals were not choosing school-mates on the basis of obvious morphological differences.

Table 10. Repeated measures ANOVA of guppy schooling behaviour, measured as the percentage of time spent with stimulus fish in choice tests. The factors are familiarity, day of testing and group. Day of testing is nested within group. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	4821.8	9.43	0.003
Group	5	295.4	0.58	0.717
Familiarity * group	5	1271.0	2.49	0.036
Day (group)	48	76.4	0.15	1.000
Familiarity * day(group)	48	950.7	1.86	0.004
Error	108	511.1		

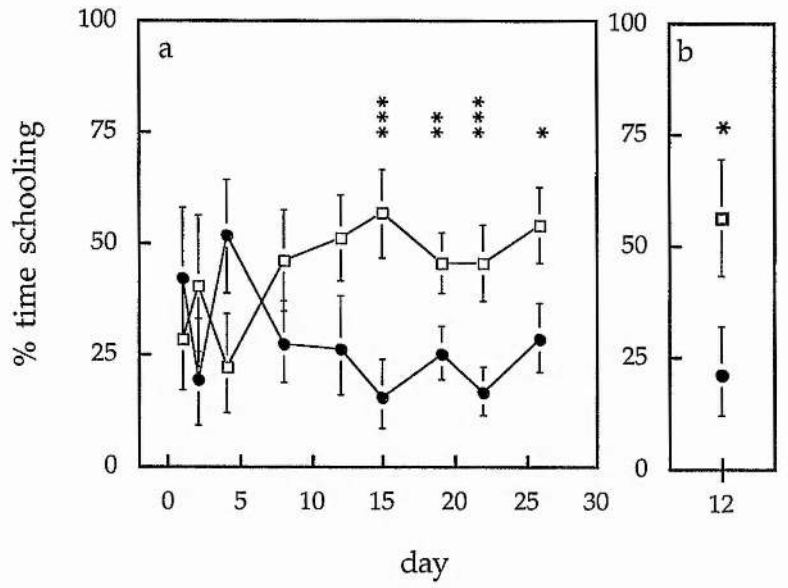

Figure 15. The percentage time a female guppy devotes to schooling with familiar guppies (open symbols) or unfamiliar guppies (filled symbols): (a) when this choice was presented repeatedly over one month; (b) after 12 days during which the test fish were not exposed to trial conditions. In all cases mean % time schooling ± 1s.e. (back transformed from arcsine % time data) is indicated (n=12). Preference for familiar schooling partners is indicated at $P < 0.05$ (*), $P < 0.02$ (**) and $P < 0.01$ (***) significance levels.

6.4 Discussion

This experiment has shown that female guppies, which had been living in groups of six, develop a schooling preference for their own tank mates after a period of about 12 days. Having attained this tendency, preferential association with familiar individuals persisted for the remainder of the one month experimental period. Females continued to school with their tank mates for a further 18 days. Evidence that preferences can be maintained over longer time scales is found in studies of threespine sticklebacks (Van Havre & FitzGerald 1988) and fathead minnows (Brown & Smith 1994). Van Havre & FitzGerald (1988) found that female sticklebacks retain preferences for natural school-mates after 10 days isolation from one another, although males did not make this distinction. For fathead minnows, however, schooling preferences for familiar conspecifics remained unchanged over an even longer time period, the former school-mates having been separated from each other for 2 months or more (Brown & Smith 1994). The level of cognitive ability which fish demonstrate seems especially impressive in light of the fact that these studies had minimised morphological differences between individual fish.

The results of this study indicate that development of familiarity among females takes some time to achieve. This is not surprising given that female guppies are uniform and cryptic in appearance and that the individuals in our tests were also size-matched and randomly allocated to the tanks. Yet there is evidence that fish may discriminate amongst particular individuals over a much shorter time frame. Milinski *et al.* (1990b) investigated the predator inspection behaviour of sticklebacks that had been kept in groups of four for a 'few' hours. They found that in all cases at least some of the individual fish had partners with whom they preferentially inspected the predator. In a separate experiment, Milinski *et al.* (1990a) showed that sticklebacks could remember the better of two inspectors after seeing each of them inspect just four times. Dugatkin & Alfieri (1991a) similarly found that guppies preferred to associate with the better of two inspectors. Three fish were placed in parallel channels and had one minute in which to settle and a further 2.5 minute in which to inspect a predator. Immediately afterwards the central fish was given a choice test in which it had the opportunity of

associating with either of its erstwhile inspection partners. In 80% of trials there was a preference for the individual that had spent most time close to the predator. This level of discrimination persisted even if partner preference was tested 4h after the inspection test, rather than at once.

Fish also maintain discrimination between school-mates who differ in competitive foraging ability (Metcalfe & Thomson 1995) or parasite load (Dugatkin *et al.* 1994). Metcalfe & Thomson (1995) placed single fish (European minnows) in the central compartment of a three-chamber aquarium. Individuals were given the choice of schooling with 3 fish of a lower competitive rank (poorer foraging ability) or higher competitive rank than themselves, the 7 fish having been housed together before the trials in order to gain experience of one another's foraging ability. The minnows showed consistent preference for the fish of low rank with which they had previously been housed for at least 6 days (Metcalfe & Thomson 1995). These tests reveal that individual fish can readily distinguish between conspecifics but do not prove that this discrimination was based on familiarity. It may be that the selection of partners during predator inspection or foraging is a form of condition-dependent recognition. Although Milinski and his colleagues (Milinski *et al.* 1990a) suggested that the quickly-established preference they saw was evidence for individual recognition, they could not exclude the possibility that the test sticklebacks had learnt to recognize the position of the better inspector rather than its actual identity. A study of sticklebacks' inspection behaviour conducted by Külling & Milinski (1992) provides support for the idea that fish assess their inspection partners on the basis of condition. They showed that sticklebacks preferred to inspect in the company of larger individuals. This is probably because large individuals are preferred by the predator and are therefore more likely to distract the predator's attention from the smaller partner (Külling & Milinski 1992).

It is also now well established that individual fish take the size of potential schooling partners into account when deciding who to associate with (Ranta *et al.* 1992b; Peuhkuri 1997) a phenomenon also observed by Parrish (1989b). Multi-species schools of atherinids, *Allanetta harringtonensis*, clupeids, *Jenkensia lamprotaenia* and

Harengula humeralis and engraulids, *Anchoa choerostoma* would often be found to segregate into small discrete single-species schools of similar size despite these species being otherwise morphologically and ecologically similar (Parrish 1989b). Birds are also found to differentiate into sub-flocks according to size and species (Powell 1974; Bertram 1978; Caraco 1980) while some flocks are structured according to a dominance hierarchy (Senar *et al.* 1990). For example, the social structure of siskin, *Carduelis spinus*, flocks has been shown by Senar *et al.* (1990) to be stable and that individual siskins joining a flock of unfamiliar birds will be subordinate to the existing flock members. It is possible that the hierarchical dominance of certain individuals over others may play a role in the schooling decisions of fish in the same way as flocking birds. The possibility that fish prefer not to school with unfamiliar conspecifics in order to avoid subordination cannot be discounted.

Since familiarity, as opposed to condition-dependent recognition, takes a number of days to develop we might expect fish to associate with particular individuals for protracted periods in the wild. There have been few attempts to investigate the schooling preferences of wild fish. (Helfman 1984), however, provides perhaps the best example of this kind of field study to date. He observed the movement and behaviour patterns of 102 individually identifiable yellow perch, *Perca flavescens*, in Cazenovia Lake, New York. The use of snorkelling equipment allowed school fidelity data to be collected in the field without disturbance to the fish, other than the initial tagging procedure. Although individual fish did co-occur, this was found to be a function of schooling tendency rather than school fidelity (Helfman 1984): individuals seemed to join and leave schools frequently.

Most studies to date have tested for schooling preferences amongst small groups of individuals and it may be that familiarity takes longer to develop, or is harder to achieve, in situations where there are more potential partners (chapter 7). However, Brown & Smith (1994) have demonstrated that olfaction plays a major role in the discrimination of familiar school-mates. Indeed, they showed that fathead minnows can distinguish familiar individuals on the basis of chemosensory cues alone. Chemosensory recognition also plays an important role in partner choice among threespine sticklebacks (Van

Havre & FitzGerald 1988) and guppies (Warburton & Lees 1996) - see chapter 8. Domestic juvenile guppies were found to prefer schooling with familiar siblings over unfamiliar siblings when mesh partitions separated the test from the stimulus fish (Warburton & Lees 1996). The mesh allowed water and therefore chemosensory cues to circulate throughout the apparatus while eliminating most visual cues available to the fish. Similarly, Van Havre & FitzGerald (1988) demonstrated that female threespine sticklebacks distinguish conspecifics on the basis of chemonsensory recognition although this ability was not apparent when the females were presented with visual cues only. The use of chemosensory cues means that fish could base their schooling decisions on the odour of their habitual school-mates and, so long as there is a shared group odour, the recognition of familiar individuals need not be constrained by group size. Nonetheless, the existing evidence for individual recognition within schools (Dugatkin & Wilson 1992; Metcalfe & Thomson 1995) implies that group size may play an important role in the acquisition of familiarity. This will be the subject of investigation in the next chapter (chapter 7).

6.5 Summary

Previous work has demonstrated that fish prefer to school with familiar individuals. This study demonstrates that schooling preferences for familiar female guppies develop gradually over a period of 12 days, and once established are maintained. This contrasts with condition-dependent recognition in which fish rapidly learn to discriminate between conspecifics on the basis of obvious morphological differences such as body size.

CHAPTER 7

Do schooling preferences for familiar fish vary with group size[1]?

The tendency of fish to recognise and preferentially associate with familiar conspecifics has been well documented in a series of laboratory experiments (e.g. Brown & Smith 1994, and see chapters 3 & 5; Magurran *et al.* 1994) and field studies (see chapter 4). While it is known that schooling preferences take time to emerge (chapter 6), it is possible that the number of potential schooling partners may also mediate these schooling preferences. If the ability of fish to distinguish between particular individuals declines gradually with increasing group size, this suggests a role for individual recognition abilities in fish. However, it may also be the case that recognition of familiar conspecifics is achieved by discrimination on the basis of a shared group characteristic. This chapter asks whether the expression of familiarity is constrained by group size.

7.1 INTRODUCTION

There are many advantages of living in groups (for example, Hamilton 1971; Wittenberger & Hunt 1985; Krebs & Davies 1993). In fish, the anti-predator (Neill & Cullen 1974; Magurran 1990a; Pitcher & Parrish 1993) and foraging (Pitcher *et al.* 1982; Clark & Mangel 1984; Krause 1993b) benefits of group living have been particularly well documented (chapter 1). It has also been suggested that anti-parasite, hydrodynamic and mate searching advantages might become available to individual members of groups (chapter 1). Moreover, the fitness advantages accrued by fish joining groups of conspecifics have recently been discovered to be further enhanced if the school is composed of familiar rather than unfamiliar fish. A recent study of fathead minnows, *Pimephales promelas*, by Chivers *et al.* (1995) has highlighted this effect by demonstrating that schools of fish

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[1]Data from part of this study have been published as Griffiths, S. W. & Magurran, A. E. (1997) Schooling preferences for familiar fish vary with group size in a wild guppy population. Proc. R. Soc. Lond. B 264, 547-551.

familiar with one another were more cohesive and increased their co-operative anti-predator behaviour compared with schools comprised of unfamiliar individuals. An increase in cohesive behaviour is known to be correlated with longer survival time, at least in the case of fathead minnows interacting with northern pike, *Esox lucius*, (Mathis & Smith 1993). It seems clear, then, than fish gain benefits by schooling with familiar conspecifics. An overview of recent studies giving evidence for partner choice by fish in the contexts of anti-predator and foraging behaviour, as well as in kin selection is presented by Dugatkin & Wilson (1993). They argue that the advantages of a cognitive ethological approach to behavioural studies of fish partner choice have been overlooked in the past and that increasing awareness of the recognition abilities of individual fish is required for successful research in the future. Further to this Dugatkin & Sih (1995) describe examples of partner choice decisions from many different taxa.

Recognition of familiar individuals may be beneficial, but under what conditions can it be achieved? The ability to discriminate familiar from unfamiliar conspecifics has been demonstrated in a wide variety of freshwater fish species including bluegill sunfish, *Lepomis macrochirus*, (Brown & Colgan 1986), threespine sticklebacks, *Gasterosteus aculeatus*, (Van Havre & FitzGerald 1988) and fathead minnows (Brown & Smith 1994). Indeed, the role of familiarity in the recognition abilities of many other fish species including Atlantic salmon parr, *Salmo salar*, (Stabell 1982; 1987; Moore *et al.* 1994); Baltic salmon parr, *Salmo salar*, (Folke *et al.* 1992); coho salmon, *Oncorhynchus kisutch*, (Quinn & Tolson 1986); juvenile Arctic charr, *Salvelinus alpinus*, (Olsén 1986); sockeye salmon, *Oncorhynchus nerka*, (Groot *et al.* 1986) and guppies, *Poecilia reticulata*, (Warburton & Lees 1996) may have been underestimated to date, but see Quinn & Busack (1985) for an exception. It is known, for example, that Atlantic salmon parr respond more strongly to the odour of urine obtained from siblings than from unrelated conspecifics (Moore *et al.* 1994). Both behavioural responses (swimming movement towards or away from the urine sample) and electrophysiological responses (measurements of voltage gradients from the surface of the olfactory epithelium) were elicited on presentation of the urine stimulus. Moore *et al.* (1994) concluded that these results provided support for the hypothesis that Atlantic salmon parr preferentially associate with siblings over unrelated conspecifics. However, because sibling groups were raised together in this study (Moore *et al.* 1994), the

possibility that the fish were discriminating between one another on the basis of familiarity, rather than relatedness could not be discounted.

Schooling preferences for familiar individuals take time to emerge. For example, bluegill sunfish associate with familiar conspecifics after three to seven days (Brown & Colgan 1986), while the tendency of female guppies to school with their tank-mates develops gradually over a period of 12 days (chapter 6, Griffiths & Magurran 1997a). Time is one factor that mediates schooling preferences for certain individuals. The number of potential schooling partners may well be another. Most of the experiments to date have looked at partner choice amongst small groups of fish in the laboratory. Brown & Colgan (1986) held groups of 5-6 bluegill sunfish together before their use in trials while Magurran *et al.* (1994) gave groups of 12-15 female guppies the opportunity to become familiar with one another before measuring their schooling preferences. In their study of domestic guppies Warburton & Lees (1996) placed newly born juveniles in groups of 8-10 individuals prior to testing. The size of natural fish aggregations, however, is often large by comparison and it is possible that the ability of fish to learn or remember the identities of particular individuals declines as there are more conspecifics to choose among. Misund (1993) measured the size of herring, *Clupea harengus*, sprat, *Sprattus sprattus*, and saithe, *Pollachius virens*, schools in Norwegian fjords and in parts of the North Sea. Remarkably, the echo location and sonar techniques used revealed that school size varied by 4 orders of magnitude (Misund 1993). Nøttestad *et al.* (1996) who measured school size of herring in south-western Norway also found the number of fish per school to be extremely variable (varying by a factor of 4). However, during times of hibernation, when herring do not forage, as many as 2 million individuals have been recorded as members of only one school (Misund 1993).

While it is hypothesised that preference for familiar school-mates gradually decreases with increasing group size, it may also be the case that association with familiar conspecifics may only be beneficial while group size remains smaller than a critical size. Beyond this point and as group size increases, the advantages of schooling with familiar fish may diminish as the 'safety in numbers' effect strengthens (Bertram 1978; Kiltie 1980; Foster & Treherne 1981; Kiltie & Terborgh 1983). The probability of being injured or eaten during a predatory attack decreases inversely with increasing school size according to a numerical dilution effect (Bertram

1978; Foster & Treherne 1981) as has been demonstrated experimentally for schooling killifish, *Fundulus diaphanus*, and the white perch predator, *Morone americana* (Morgan & Godin 1985). In large schools, therefore, where the probability of attack for each school member is low, individuals may trade-off the cost of searching for familiar conspecifics against the benefits of activities such as foraging.

Alternatively, apparent recognition of familiar conspecifics may in fact be recognition of a characteristic shared by all group members (e.g. similarity of morphology or chemosensory cues). If this is true then group size *per se* may play little role in determining schooling preferences. In order to test the hypothesis that the tendency of fish to school with familiar individuals will be inversely related to the size of group in which they naturally occur, the behaviour of a wild population of guppies in Trinidad was examined.

7.2 METHODS

Study Species and Field Site

Guppies occur widely in Trinidad. Part of their range includes intermittent rivers such as those found in the upper reaches of the Northern Range Mountains (see chapter 2, figure 3). In streams like the Upper Tunapuna, guppies can be confined to isolated pools for several months during the dry season (figure 16). The number of guppies within each pool in this stream is highly correlated during the dry season with surface area; pool sizes fall within the range of $1m^2$ to $10m^2$ (B. H. Seghers & A. E. Magurran, unpublished data). A guppy in the Upper Tunapuna will thus find itself interacting with a variable number of fish, depending upon the size of pool in which it happens to be located. This experiment examines the schooling preferences of female guppies. Although male guppies are brightly coloured and polymorphic, females are cryptic in appearance, and to the human eye at least, are not readily distinguishable. However, since female guppies have a higher schooling tendency than males, and invest more in anti-predator defence (Magurran & Seghers 1994b) they stand to make considerable gains from partner preferences even if they do find it more challenging to recognise one another.

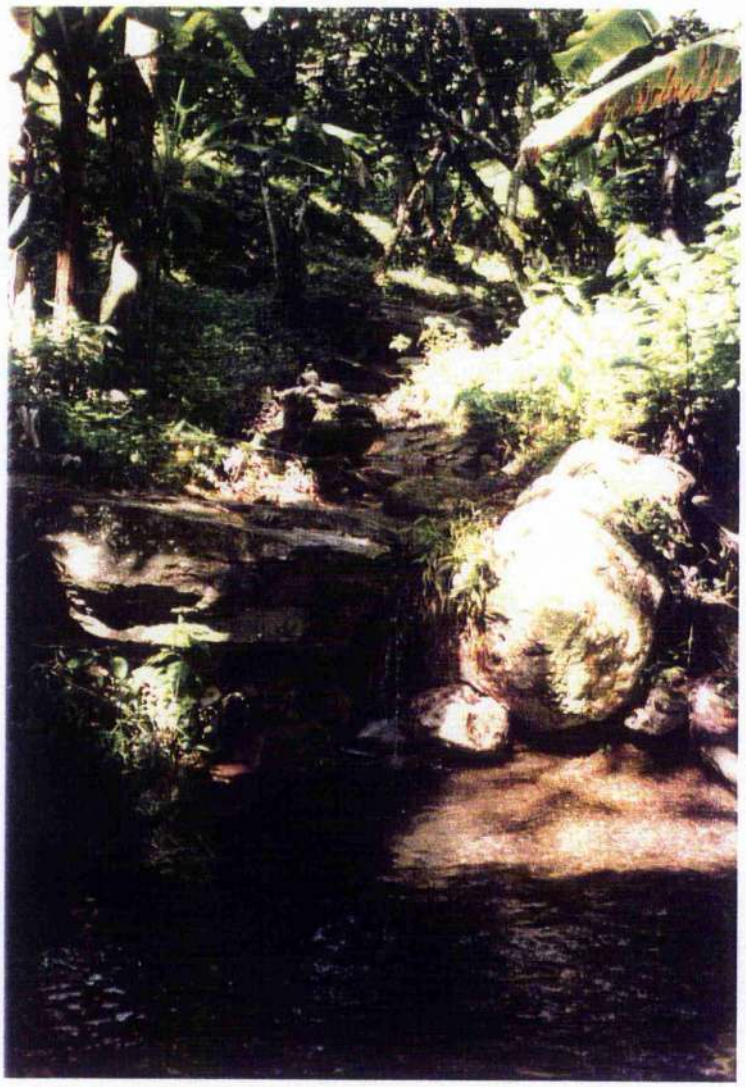

Plate 9. The Upper Tunapuna River, northen mountain range, Trinidad during March 1996. The pool at the bottom contained 194 female gupppies

The Upper Tunapuna is a low predation system (Magurran & Seghers 1994c) where guppies co-occur with the cyprinodont, *Rivulus hartii*. The swamp eel *Synbranchus marmoratus* is also present. Like many other low predation habitats in Trinidad, this guppy population is female biased. At the time of this study there was a 2:1 sex ratio in favour of females in the Upper Tunapuna (B. H. Seghers & A. E. Magurran, unpublished data).

The study was conducted in March 1996. Seven discrete pools, containing from 8 to 194 adult females (B. H. Seghers & A. E. Magurran, unpublished data), were selected for the investigation. Some of the pools in the 20 pool section of the river contained too few fish for the purposes of this experiment, while other pools were contiguous and effective group size could not be precisely determined. By choosing isolated pools it was fairly certain that the female guppies in them had coexisted for about 3 months during the dry season. Laboratory tests indicate that familiarity is acquired over a matter of days (chapter 6) and therefore had ample time to develop in these isolated pools. For this reason we designated females in a given pool as 'familiar'. The study was comprised of two parts: an investigation of group size on schooling preference (where group size refers to the total number of females in a pool) and a control to examine the effects of the batch marks used to distinguish familiar from unfamiliar individuals.

(a) Experiment

A small natural pool (110 cm max. length x 60 cm max. width x 5 cm max. depth), isolated from others in the system, was used as the observation arena. No other fish, of any species, occurred in this pool. Four equally sized familiar females, randomly chosen from one of the seven groups and 4 equally sized unfamiliar female guppies, from a different pool, were gently transferred to the observation pool. Unfamiliar fish (i.e. ones that were unknown to the test females, though not necessarily from each other) were given a small alcian blue mark on their caudal peduncle. This meant that they could be easily distinguished from the familiar females. Marked fish were allowed to settle for 1 hour before the familiar fish were introduced. All fish were then given a further 20 min settling time before observations were made. Two familiar fish were observed for 15 min in succession. The time that each focal fish spent schooling with a familiar or

a unfamiliar nearest neighbour was measured. Total schooling time was also recorded. For the purposes of this experiment a focal fish was defined as schooling if it was within 3 body lengths of another female. This can be regarded as a conservative definition of schooling as (Pitcher 1983) considered 4 body lengths to be an appropriate distance by which school members would be maximally separated. At the end of the trial the total length of each fish was measured. Observations were made on at least 4 and in some cases 6 or 8 individuals from each of the seven pools investigated (figure 17). No fish was tested more than once.

(b) Control

The aim of the control experiment was to ascertain whether the marking procedure influenced schooling preferences. Eight familiar guppies, i.e. females originating from the same pool, were placed in the observation arena. Four randomly selected individuals were marked in the usual way. All fish were allowed to settle for 1 hour before the schooling behaviour of marked and unmarked focal fish was recorded as described above. The trial was repeated four times, so that observations were made on eight individuals in total.

Data Analysis

Preferences for familiar and unfamiliar schooling partners were expressed as percentage of total time schooling in order to account for variation in schooling tendency between groups. Since the behaviour (time spent with familiar fish) of the two successive focal fish in each trial did not differ significantly (one-way ANOVA: $F_{1,86} = 5.69 \times 10^{-12}$, $P > 0.99$, mean and standard deviation for each focal fish 50.0, 50.0 and 15.51, 14.87 respectively), both data points were used in the following analysis. A two-way general linear model (glm) investigated the effect of familiarity and group size on partner choice during schooling. A glm was chosen as it allows analysis of non-orthogonal data (McCullagh & Nelder 1983).

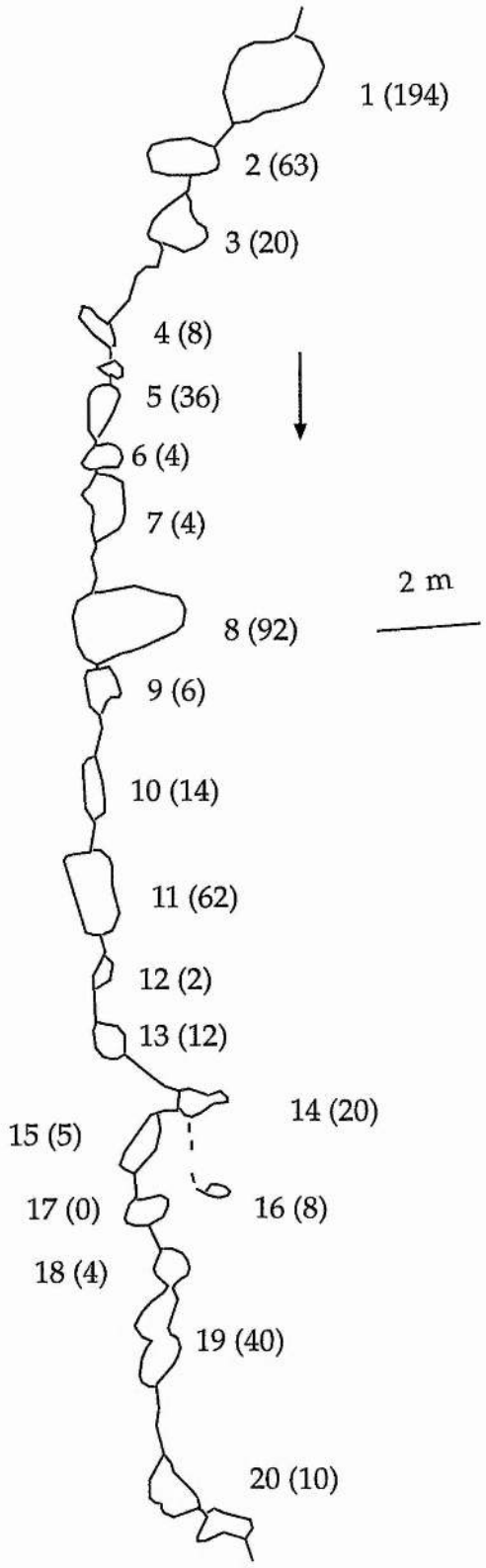

Figure 16. Map of The Upper Tunapuna River, Trinidad, during March 1996 (dry season) showing the identification number of each pool in a series of 20. Figures in brackets indicate the number of female guppies in each pool. Reproduced with the permission of B. H. Seghers and A. E. Magurran. Arrow shows direction of water flow.

7.3 RESULTS

The analysis confirmed the hypothesis that wild female guppies prefer to school with familiar individuals ($F_{1,74}$ = 148.35, P<0.001, table 11). The interaction between familiarity and group size was highly significant ($F_{6,74}$ = 15.20, P<0.001, table 11) indicating that schooling preferences for familiar fish decreased as group size increased (figure 17). This suggests that the recognition ability of guppies is constrained by the number of individuals with whom they interact. Familiar and unfamiliar guppies did not differ in size ($F_{1,138}$ = 1.68, P = 0.198), their mean total lengths being 27.5 mm (± 1s.e. = 0.3) and 28.0 mm (± 1s.e. = 0.3) respectively.

The control demonstrated that schooling decisions were not influenced by the marking procedure. Female guppies equally familiar with one another did not demonstrate a preference for schooling with either marked or unmarked fish ($F_{1,12}$ = 3.19, P = 0.099). The total lengths of marked and unmarked fish did not differ either (one-way ANOVA $F_{1,14}$ = 0.01, P = 0.93). There is no reason to suspect, therefore, that the preferential association with familiar fish found in the main experiment can be attributed to preferences for the presence or absence of alcian blue marks.

Table 11. Two-way general linear model of female guppy schooling behaviour, measured as the percentage of time spent with stimulus fish during schooling. The factors are familiarity and group size. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	10065.2.8	148.35	0.000
Group size	6	0	0	1.000
Familiarity * group size	6	1031.2	15.20	0.000
Error	74	67.8		

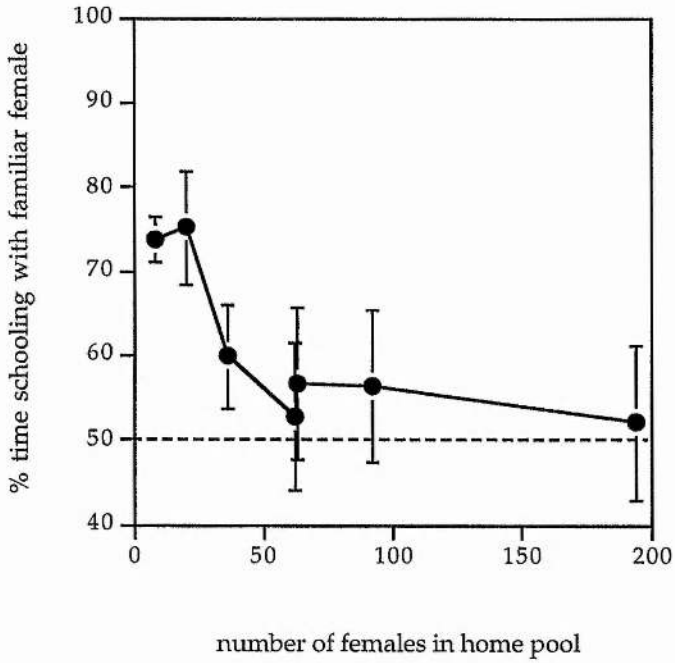

Figure 17. Relationship between group size and schooling preferences for familiar females. The graph shows the mean time (± 95% confidence limits) that focal individuals spent schooling with familiar females. Schooling time with familiar fish is expressed as a percentage of total schooling time for that individual. The hatched line represents the point at which equal time is spent schooling with familiar and unfamiliar schooling partners. The total number of females per pool and the number of fish tested from that pool were: **8** females in pool & n = 4 females tested; **20** & n = 6; **36** & n = 8; **62** & n = 6; **63** & n = 6; **92** & n = 6; **194** & n = 8.

7.4 DISCUSSION

This study reveals that wild female guppies which have been living together for a number of months prefer to school with familiar partners. This result indicates that wild schools may not be random assemblages of conspecifics and that familiarity, based on past interactions, can influence the choice of schooling partner. Indeed, in the case of fish from the smaller groups, these preferences are even stronger than those documented previously for guppies in the laboratory (chapter 6, Magurran *et al.* 1994). Guppies from the Lower Aripo and Lower Turure River populations (both high predation sites) spend approximately 60% of total time schooling with familiar conspecifics in a bottle choice test (Magurran *et al.* 1994). Under similar experimental conditions Lower Tacarigua River guppies (also a high predation site) were shown to associate with familiar fish for up to 57% of total schooling time (chapter 6), while in this investigation the equivalent figure for female Tunapuna guppies (a low predation site) was as much as 75%.

It is possible that part of the reason for this difference in schooling preference may be due to differences in schooling tendency between guppies originating from different populations. However, fish from high predation sites spend a greater proportion of time schooling than fish from low predation sites (Seghers 1974b; Magurran & Seghers 1994b). Moreover, the benefits of choosing familiar school-mates is expected to be greatest in high predation sites (Chivers *et al.* 1995). Future work (controlling for group size effect) may yet reveal fish from high risk sites to be more adept at recognising potential schooling partners. Such fish do, after all, have a greater predisposition to refine their anti-predator behaviour as a consequence of early experience (Magurran 1990b; Huntingford & Wright 1993). However, since guppies in high predation sites are rarely restricted to isolated pools they may encounter more individuals during their daily activities than Upper Tunapuna fish.

In the absence of a high threat of predation what other advantages might fish obtain from schooling with familiar individuals? An adaptive explanation for the behaviour of Upper Tunapuna females is that feeding benefits accrue from schooling with familiar pool-mates. Familiar fish may be able to forage more efficiently than unfamiliar ones by avoiding

exploited food patches and by knowing the competitive abilities of their foraging partners (Metcalfe & Thomson 1995). If this were true, familiar individuals would gain direct feeding advantages and, in the case of females, the indirect benefits of increased fecundity which is related to body mass (Reznick *et al.* 1990).

This investigation also demonstrates that schooling preferences for familiar individuals are mediated by group size. Females originating from small groups are able to recognise and preferentially associate with familiar conspecifics. However, as group size increases, the behaviour is progressively lost and females choose their neighbours at random. The precise point at which familiarity ceases to influence behaviour cannot be fixed. Although a range of group sizes were tested, these were chosen from ones made available by nature and could not, therefore, be predetermined. Yet, the trend is clear and there is a pronounced interaction between familiarity and group size. Examples of this phenomenon in other animals are rare. Pagel & Dawkins (1997) modelled the relationship between group size and the presence of dominance relationships amongst individuals so as to be able to predict the welfare implications of housing domestic hens, *Gallus gallus domesticus*, in large groups. Only small groups of hens recognised each other and formed dominance hierarchies because only under these conditions of high probability of repeatedly meeting the same individuals were the costs of recognition balanced by the benefits of avoiding contests between individuals (Pagel & Dawkins 1997). The authors, however, could not confirm that individual recognition of opponents rather than status category recognition would be required by hens in order to meet with the predictions of the model (Pagel & Dawkins 1997).

Why do partner preferences for familiar fish diminish in larger groups? One possibility is that the choice of school-mates is based on individual recognition. If this were the case then females in the largest pool would have to learn the identities of almost 200 individuals in order to recognise them all as schooling partners. Neural capacity is one factor which constrains the recognition abilities of animals forming groups (Jaffe & Perez 1989; Dunbar 1992; Jaffe & Chacon 1995). In primates, the extent of social networks formed by each of 38 genera described by Dunbar (1992) is limited by neural capacity so that a significant relationship exists between relative neocortex volume and size of the social group in which the animals

typically live (Dunbar 1992; Dunbar 1995). Some social insects also demonstrate a positive trend between group size and sophistication of their neural apparatus (Beckers *et al.* 1989; Jaffe & Perez 1989; Jaffe & Deneuberg 1992; Jaffe & Chacon 1995). The most social (those which form the largest colonies) of 13 species of Formicidae ants were found by Jaffe & Perez (1989) to have both the most complex chemical communication systems and the most highly developed corpora pedunculata and olfactory lobes. It seems plausible that social relationships based on individual recognition will be constrained by brain size in fish too. It may be that female guppies can recognise a certain number of individuals and that this number will remain constant irrespective of the group size in which they naturally occur. Thus, even in large pools, females might preferentially associate with certain individuals. The possibility of selecting sub-groups of females was deliberately avoided by choosing fish, at random, from different sections of a pool.

A previous study of schooling fidelity in wild fish (Helfman 1984) found little tendency towards associations among particular individuals. On the other hand, a study of juvenile coho salmon allowed to swim in the near-natural environment of an artificial channel measuring over 30m long and 4.5m wide established that distribution of 778 individuals among schools was significantly different to that which would be expected if the fish were choosing schooling partners at random (Quinn *et al.* 1994). However, these preferred schooling partners were related individuals suggesting kin recognition rather than individual recognition to be the most parsimonious explanation of this behaviour (Grafen 1990).

Discrimination between conspecifics on the basis of a shared odour, or some other shared group characteristic, eliminates the requirement for learning individual identities. Indeed, olfactory stimulation is a well documented cue used by fish during homing behaviour (reviewed by Hasler & Scholz 1983) and is also implicated in partner choice in fish (e.g. Brown & Smith 1994, and see chapter 8). In the case of fathead minnows Brown & Smith (1994) have demonstrated a strong role for olfaction in the preference for familiar school-mates while Quinn & Busack (1985) have revealed the ability of juvenile salmon to preferentially associate with siblings on the basis of chemosensory cues. Furthermore, female threespine sticklebacks choose familiar school-mates over unfamiliar fish when chemosensory cues are provided, but are unable to accomplish this

discrimination when only visual cues are made available (Van Havre & FitzGerald 1988). It is also known that condition-dependent recognition, that is the recognition based on traits such as body size or competitive ability or inspection tendency, is acquired more quickly (Milinski *et al.* 1990a; Milinski *et al.* 1990b; Ranta & Lindström 1990; Dugatkin & Alfieri 1991a; Metcalfe & Thomson 1995) than recognition based on familiarity alone (chapter 6). Despite this, the relationship between schooling preference and group size would not have been observed at all if the female guppies in this study could recognise fish from their own pool on the basis of one or more group characteristics.

It could be argued that marked fish have a different schooling tendency to unmarked fish. A laboratory experiment was conducted in order to demonstrate that time spent schooling by marked and unmarked females does not differ ($F_{1,19}$ =0.55, P =0.467). The procedure closely followed the 'bottle method' described by Magurran *et al.* (1994) and chapter 6. Each of twenty Upper Tunapuna females (10 of which were marked one hour previously, the other 10 remaining unmarked) were allowed to choose between either an empty transparent bottle or an identical bottle containing 4 females. Total length (mm) of marked and unmarked fish did not differ ($F_{1,19}$ = 0.57, P = 0.462).

Kinship is another factor that could explain why partner preferences vary with group size and it is possible that females in small pools are more closely related than those in large ones. This scenario cannot be excluded completely although a laboratory experiment described in chapter 8 seems to suggest that familiarity plays a more important role in the schooling decisions of guppies than relatedness. However, it is important to note that the flow between the pools in the Upper Tunapuna increases considerably during the wet season (May to December) and there is opportunity for fish movement during much of the year.

Many fish are now understood to have the cognitive ability to distinguish kin from non-kin, although work to date has concentrated on salmonids. For example, Quinn & Busack (1985) demonstrated that juvenile coho salmon prefer water conditioned by both familiar and unfamiliar siblings over non-siblings. Similarly, Brown & Brown (1992) found that both juvenile Atlantic salmon and rainbow trout, *Oncorhynchus mykiss*, can discriminate kin from non-kin, preferring water conditioned by

kin in a two-choice tank. Recently, work by Warburton & Lees (1996) has demonstrated that kin discrimination may also be possible for a domestic strain of guppy. However, the authors were unable to provide assurance that familiarity was controlled for in this study and it is therefore possible that both kinship and familiarity affected the schooling decisions of these domestic guppies. Although it is clear that fish are able to distinguish relatives from unrelated conspecifics, and despite the apparent advantages to an individual of choosing to school with kin (Blaustein *et al.* 1987), there is little evidence to date to support the idea that wild schools are composed of related individuals (Avise & Shapiro 1986; Naish *et al.* 1993).

7.5 Summary

The ability of fish to recognise and preferentially associate with familiar conspecifics has been well documented in a series of laboratory experiments. This study investigates the schooling preferences of wild female guppies, *Poecilia reticulata*, in the Upper Tunapuna River in Trinidad and confirms that they do indeed prefer to associate with familiar individuals. The guppies in this river occur in a series of pools that become isolated during the dry season. These fish interact solely with other individuals in their pool for periods of several months at a time and thus have ample opportunity to become accustomed to one another. Our study also reveals that the tendency of female guppies to school with familiar fish declines as the group size in which they naturally live increases. Preferences are strong when there are small numbers of females in a pool, but diminish thereafter. This indicates that the expression of familiarity is constrained by group size.

CHAPTER 8

The role of kinship in the recognition of school-mates: how important are visual and chemosensory cues?

8.1 Introduction

The remarkable discriminatory abilities of fish allow individuals to recognise conspecifics and choose amongst them on the basis of past experience. Threespine sticklebacks, *Gasterosteus aculeatus*, prefer individuals with whom they have successfully performed predator inspection behaviour in the past (Milinski *et al.* 1990a), while Dugatkin & Wilson (1992) have shown that bluegill sunfish, *Lepomis macrochirus*, choose schooling partners on the basis of foraging success. Furthermore, condition independent recognition (see chapter 6) makes possible discrimination between conspecifics without requiring a cue such as foraging ability to indicate some aspect of individual condition. Many freshwater fish recognise individuals on the basis of familiarity including bluegill sunfish (Brown & Colgan 1986; Dugatkin & Wilson 1992), threespine sticklebacks (Van Havre & FitzGerald 1988), paradise fish, *Macropodus opercularus*, (Mikloski *et al.* 1992), fathead minnows, *Pimephales promelas*, (Brown & Smith 1994) and guppies, *Poecilia reticulata*, (Magurran *et al.* 1994). This preference confers anti-predator (Chivers *et al.* 1995) and foraging (Metcalfe & Thomson 1995) benefits upon schooling fish.

Only a few cases exist of wild fish preferentially associating with natural school-mates. Nevertheless, the evidence is strong enough to suggest that naturally occurring schools are composed of individuals which are familiar with one another. For example, guppies from the Tunapuna River, Trinidad were found to prefer their natural school-mates when Griffiths & Magurran (1997b, see chapter 7) observed partner choice decisions of wild female fish in the wild. European minnows, *Phoxinus phoxinus*, observed in the naturalistic conditions of a fluvarium prefer their natural school-mates (chapter 4, Griffiths 1997). Brown & Smith (1994) observed fathead minnows in the laboratory, but were also able to determine that these fish prefer their natural, familiar, school-mates. It seems, then, that schools of fish in the wild may be composed of non-

random assortments of individuals. However, the possibility that fish captured together in schools are related to one another and that recognition is being achieved on the basis of kinship instead of, or as well as, familiarity cannot be discounted.

Kinship is known to affect the partner choice decisions of fish, and the preference by individuals for related conspecifics has been especially well described in anadromous species such as Atlantic salmon parr, *Salmo salar*, (Stabell 1982; 1987; Moore *et al.* 1994); Baltic salmon parr, *Salmo salar*, (Folke *et al.* 1992); coho salmon, *Oncorhynchus kisutch*, (Quinn & Tolson 1986); juvenile Arctic charr, *Salvelinus alpinus*, (Olsén 1986); sockeye salmon, *Oncorhynchus nerka*, (Groot *et al.* 1986) and rainbow trout, *Oncorhynchus mykiss*, (Brown & Brown 1992). Atlantic salmon parr elicit behavioural as well as electrophysiological responses to support the hypothesis that these fish preferentially associate with siblings over unrelated conspecifics (Moore *et al.* 1994). However, Moore *et al.* (1994) and other similar studies may not have taken into consideration the possibility that kin housed together from time of fertilisation of eggs until the experiment was undertaken may have also become familiar with one another during this period. Indeed, Courtenay *et al.* (1997) found that common rearing increased the preference of juvenile coho salmon for their siblings. A more parsimonious explanation for the results obtained by Moore *et al.* (1994) may be that the salmon demonstrate the ability to distinguish conspecifics on the basis of familiarity rather than kinship (Grafen 1990). Although some studies do control for familiarity effects (e.g. Quinn & Busack 1985), clearly the degree to which relatedness and familiarity play a role in the partner choice decisions of fish is yet to be fully elucidated.

The mechanisms by which fish achieve discrimination among conspecifics has been suggested to include chemosensory (Hara 1992) auditory (Myrberg & Riggio 1985) visual and lateral line recognition (Partridge & Pitcher 1980). Investigations of midas cichlid, *Cichlasoma citrinellum*, centarchid fish (rock bass, *Ambloplites rupestris*; bluegill sunfish and pumpkinseed sunfish, *L. gibbosus*) and threespine sticklebacks' schooling decisions have all found one of the most important sensory modalities to be chemosensory recognition (Barnett 1982; Brown & Colgan 1986; Van Havre & FitzGerald 1988; Hara 1992). Van Havre & FitzGerald

(1988) found that female threespine sticklebacks prefer to school with familiar conspecifics when presented with chemosensory cues to the identity of the potential schooling partners. It may be that discrimination on the basis of only chemosensory cues allows individuals to gain the benefits of discrimination (Chivers *et al.* 1995; Metcalfe & Thomson 1995) while remaining free of the constraints of maintaining visual contact with school-mates. On the other hand, the transfer of chemosensory cues is relatively slow compared to vision, and in an aquatic environment chemical cues become diffuse and are swept away by prevailing currents. Vision is known to be one of the primary senses by which fish recognise each other. School cohesion is commonly reduced during darkness (Fréon & Misund 1998) and schooling is maintained even if other sensory information is blocked by cutting the afferent lateral line nerves (Cahn 1972; Pitcher 1979; Partridge & Pitcher 1980). Cichlid parents (Myrberg 1975) and fry (Hay 1978) recognise each other by using visual cues although recognition on the basis of chemoreception is also possible. Barnett (1977) suggested that this sensory modality may be favoured by cichlids while under conditions of low light intensity, or murky water.

To what extent might the preference by wild fish for their natural (familiar) school-mates (Brown & Smith 1994; Griffiths & Magurran 1997b) be attributed to relatedness? Studies of relatedness among the members of wild schools have provided equivocal results to date (Ferguson & Noakes 1981; Avise & Shapiro 1986; Dowling & Moore 1986; Naish *et al.* 1993). Some fish species are unlikely to form schools of related individuals. For example, the juveniles of those species with broadcast spawning or planktonic larvae are unlikely to be reunited within schools during adulthood. Avise & Shapiro (1986) found little evidence for greater relatedness within than between groups of the serranid reef fish, *Anthias squamipinnis*, which disperses its eggs during a pelagic phase. However the reproductive biology of some fish may favour the prolonged association of kin. The threespine stickleback is one such example. Males build nests where eggs and then fry are cared for and furthermore, most eggs will have been fertilised by this carer (Rico & FitzGerald 1991; Rico *et al.* 1991). Therefore not only will most of the brood be related but the juveniles have the opportunity to choose to stay together once they leave the nest. It might be predicted then, that naturally occurring schools of adult

sticklebacks are related to one another although evidence of this has yet to be produced.

The guppy is an ovoviviparous species in which juveniles school from birth. The relatedness levels within schools may therefore be relatively high, and kinship may have a strong role to play in the schooling decisions of individuals. A recent study by Warburton & Lees (1996) provided some evidence of kin discrimination in a domestic guppy strain, although familiarity effects were not strictly controlled (table 1 and chapter 5). This study aims to investigate the relative roles which familiarity and kinship play in the schooling decisions of the guppy by asking the following questions. First, do guppies prefer familiar to unfamiliar kin? It is hypothesised that the answer to this question will confirm previous work which has demonstrated a preference by guppies for their familiar conspecifics. Second, the question of whether guppies prefer unfamiliar siblings to unfamiliar unrelated conspecifics will be addressed in order to test the role which kinship plays in partner choice decisions. Test fish will be given the opportunity to make these choices with the aid of either visual or chemosensory cues in an investigation of the mechanisms of recognition.

8.2 Methods

Experimental animals

The guppies used in this study were descendants of fish collected in the wild from the Lower Tacarigua River, Trinidad. In order to answer the questions outlined in the introduction, groups of fish which were related and had been raised together and groups of fish which were related but had not been raised together were required. Kin groups were produced by taking females from the laboratory stock population and allowing them to mate with different males. Each pair was placed within an opaque plastic bottle (15 x 8 x 25cm) containing 2l water, an air stone and a clump of Java moss, *Vesicularis dubyana*. The fish were fed daily with Tetramin™ and kept at 24.9°C (mean ± s.d. = 2.6) and on a 12 h light regime (November 1996 - April 1997). Males were removed after three days. Females remained isolated and were checked daily thereafter for the birth of broods. Broods of 12 juveniles or more (n=18 broods of sufficient size) were separated into

2 groups of 6 or 7 individuals each. Surplus juveniles were returned to the stock populations. This gave 36 groups (kin reared together and kin reared apart). Juveniles collected from each female in this way were assigned full sibling status (or at least shared maternal genes) and were certainly more closely related within than between broods.

Each group of juveniles was housed in an opaque tank under the same regime as described above and were visually and olfactorily isolated. Juveniles which shared a tank were designated 'familiar' fish whereas juveniles in different tanks were known as 'unfamiliar' fish. The juveniles were fed Tetramin™ baby food daily for one week, then adult flake food for the remaining 2.5 weeks before trials were undertaken. The fish were tested whilst juveniles to prevent any between-group variance in sex-ratio from biasing schooling decisions. While female guppies are cryptically coloured and appear, to the human eye at least, to be very similar, discrimination between males is more easily achieved as they can be recognised individually by their colour patterns. At three and a half weeks of age the fish could not be sorted according to sex. Males had not developed colour patterns, nor gonopodia. At no time during the trials were sigmoid displays or thrusting attempts observed.

(a) Familiarity experiment

This experiment tested the hypothesis that juvenile guppies would preferentially associate with familiar siblings to unfamiliar siblings when given visual or chemosensory cues only. Individual schooling tendency was measured using a similar procedure to that adopted by Magurran *et al.* (1994) after Keenleyside (1955), also see chapter 6. Two plastic 1l bottles (22 cm high) were positioned 6 cm from either end of a test tank (90 x 32 x 20 cm water depth) see figure 14. The bottles were either clear to allow visual communication only or opaque and punctured to allow chemosensory (olfactory and gustatory) communication only. Before a trial, four juveniles from each of two groups were placed in two separate bottles. It was intended that a test guppy from each of the 2 groups per brood (n =2x18 =36) had the opportunity to associate with either the familiar siblings (from its own group) and unfamiliar siblings (from the different group, but same brood) or to remain solitary. After 30 min settling time, the test fish was released into the centre of the test aquarium. Water in the experimental tank was changed after chemosensory cue trials.

Schooling behaviour was measured by recording the length of time the test fish spent within 10 cm of each bottle. Marks drawn on the glass of the test aquarium denoted these 10 cm sections and a third line denoted the centre of the tank. The mean number of times which this central line was crossed by the test fish was measured. Trials lasted 15 min. Each fish was tested twice, the position of the bottles being swapped between trials in order to account for any side-bias in schooling tendency. Stimulus fish were not subsequently used as test fish, although in some cases test fish may have subsequently been used as stimulus fish. It is also possible that stimulus fish were used both in the visual cues as well as the chemosensory cues trials. The total length and wet weight of the fish in each group was measured at the end of the experiment in order to confirm that fish size did not vary significantly between tanks.

(b) Kinship experiment

The hypothesis was tested that juvenile guppies would preferentially associate with unfamiliar siblings as opposed to unfamiliar unrelated conspecifics when given visual or chemosensory cues only. Test juveniles from each of the 2 groups per brood (n= 2x18 =36) chose between associating with unfamiliar siblings (different group, same brood), unfamiliar unrelated conspecifics (different group, different brood) or to remain solitary in the same way as described above. Test fish had not been used as test fish in previous trials.

Data analysis

It was noted, during the course of the experiment, that some guppies simply swam to one side of the experimental tank, remaining near only one school of fish for the remainder of the trial without sampling the stimulus fish in the second bottle. It is possible that the small size of the juveniles in comparison to the apparatus may have meant that some individuals were unaware that a choice of schooling partners was available to them. The mean number of times which the line denoting the centre of the tank was crossed by the test fish during the 15 minute trials was 4.88 (± s.d. = 4.06) and 5.11 (± s.d. = 4.32) for familiarity and kinship experiments respectively, lower than the figure 7.29 (± s.d. = 4.22) noted for adult guppies exposed to the same set-up (chapter 7, Griffiths & Magurran 1997b) and 5.53 (± s.d. = 5.14) (chapter 6, Griffiths & Magurran 1997a). For

this reason it was decided that trials where test fish had not swum across the central line were to be excluded from further analysis. In the familiarity experiment, 30 of 72 trials were therefore excluded, and in the kinship experiment, 19 of 72 trials were excluded. In the case of test fish which had crossed the central line in both of its two trials, average values of time schooling with stimulus fish were used for analyses.

Percentage of time spent with stimulus fish (of total schooling time) was used in the analyses to account for differences in schooling tendency between juveniles. Proportion data were Arcsine transformed (Sokal & Rohlf 1995). Two-factor general linear models (glm) tested the effects of familiarity and cue or kinship and cue on this behaviour. A glm was chosen as it allows analysis of non-orthogonal data (McCullagh & Nelder 1983).

8.3 Results

(a) Familiarity experiment

Juvenile guppies preferred to school with familiar kin over unfamiliar kin ($F_{1,62}$ = 4.53, P = 0.037, table 12), spending 59.09% and 40.91% of total time schooling with familiar and unfamiliar stimulus fish respectively (figure 18). Sensory cue had no effect on time spent schooling ($F_{1,62}$ = 0.00, P = 1.00, figure 18), preferences for familiar conspecifics were maintained when either visual or chemosensory cues were presented. The interaction effect between familiarity and cue was not significant (table 12).

(b) Kinship experiment

Neither kinship nor sensory cue affected schooling decisions of the guppies (figure 19). Contrary to expectations, guppies did not preferentially associate with kin: juvenile fish spent equal amounts of time associating with unfamiliar siblings and unfamiliar unrelated conspecifics ($F_{1,64}$ =0.23, P = 0.630, table 13). The presentation of visual or chemosensory cues did not affect these partner choice decisions ($F_{1,64}$ = 0.00, P =1.00, table 2, figure 19). The interaction effect between kin and cue was not significant (table 13).

Table 12. Two-way glm of guppy schooling behaviour, measured as the percentage of schooling time spent with stimulus fish in choice tests. The factors are familiarity and sensory cue. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Familiarity	1	1806.9	4.53	0.037
Cue	1	0	0	1.0
Familiarity * cue	1	2.7	0.01	0.934
Error	62	398.8		

Table 13. Two-way glm of guppy schooling behaviour, measured as the percentage of schooling time spent with stimulus fish in choice tests. The factors are kinship and sensory cue. Data were arcsine transformed. * indicates an interaction between factors.

Source	*df*	Mean square	*F*	*P*
Kinship	1	66.7	0.23	0.630
Cue	1	0	0	1.0
Kinship * cue	1	47.0	0.16	0.686
Error	64	285.2		

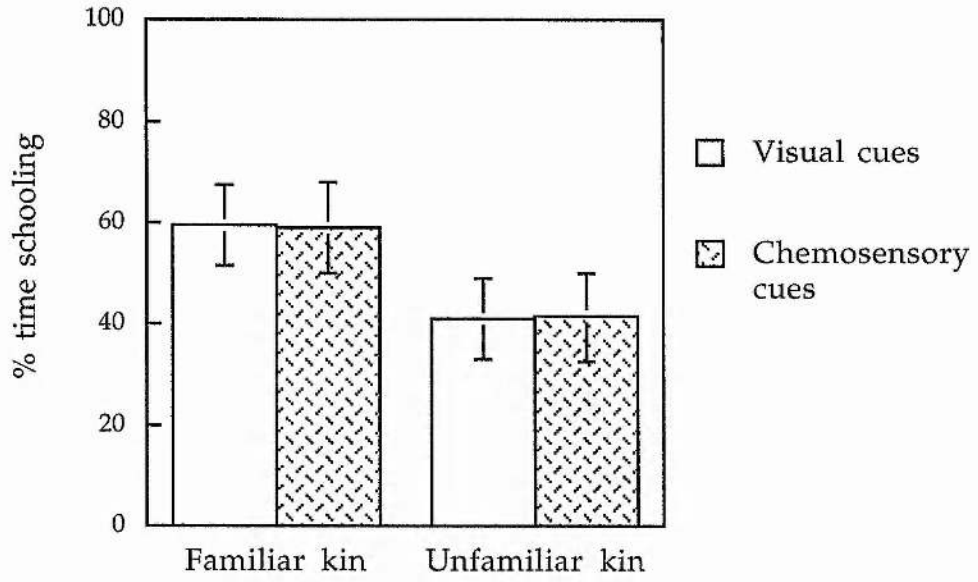

Figure 18. Time spent schooling (as proportion of total time schooling) by juvenile guppies with familiar or unfamiliar kin in the presence of either visual or chemosensory cues. Means ± 1s.e. are given for a total of 33 fish tested (visual cues, n=17; chemosensory cues, n=16). Arcsine data are back transformed.

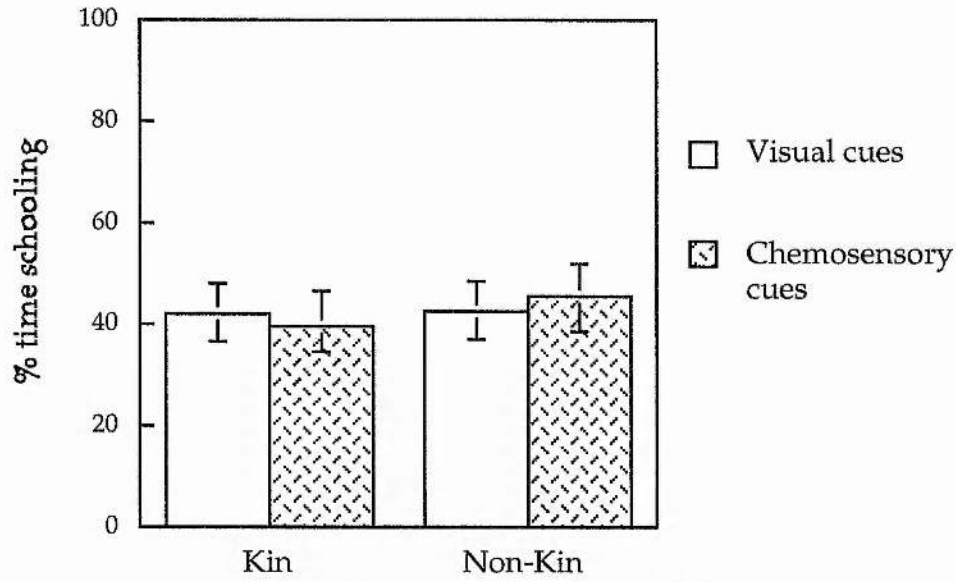

Figure 19. Time spent schooling (as proportion of total time schooling) by juvenile guppies with unfamiliar kin or unfamiliar non-kin in the presence of either visual or chemosensory cues. Means ± 1s.e. are given for a total of 34 fish tested (visual cues, n=17; chemosensory cues, n=17). Arcsine data are back transformed.

8.4 Discussion

Familiarity experiment

These data confirm that guppies are able to recognise familiar conspecifics and choose to school with them when given the opportunity to do so. Juvenile guppies spend on average 59% of total schooling time with familiar siblings. The potential effect of relatedness on partner choice decisions was controlled during this experiment so that kinship cannot have effected an increased preference for familiar conspecifics. It seems, therefore, that previous studies of the role of familiarity in guppy schooling decisions (chapters 3, 6 and 7) have provided true indications of the preference for familiar counterparts. In chapter 6, adult females which had been housed together for 24 days were found to spend approximately the same amount of time (65%) with familiar school-mates as demonstrated by this experiment. Similarly, in an almost identical experimental set up, female guppies from the Lower Aripo and Lower Turure River populations spent 60% of schooling time with familiar fish (Magurran *et al.* 1994).

Juvenile guppies were able to distinguish between potential school-mates on the basis of both visual and chemosensory recognition cues. These two sensory modalities provide enough information for guppies to recognise familiar group members. It is therefore possible that discrimination was being achieved on the basis of shared group characteristics. In other fish species, recognition and preferential association with familiar conspecifics has been achieved only with chemosensory but not visual cues e.g. threespine sticklebacks (Van Havre & FitzGerald 1988) and fathead minnows (Brown & Smith 1994). However, there are no known examples of preference for familiar conspecifics being achieved only with the aid of visual but not chemosensory cues. Wild guppies are not the only fish where chemosensory and visual discrimination is proven. Bluegill sunfish (Dugatkin & Wilson 1992), threespine sticklebacks (Peeke & Veno 1973) and a domestic strain of guppy (Warburton & Lees 1996) are also able to make this distinction. Colgan (1983) provides a review of visually based recognition. It would be interesting in future to assess whether simultaneous provision of both

visual and chemosensory communications between guppies would result in an even greater degree of preference for familiar individuals.

Is the sensory modality used by an individual to discriminate school-mates dependent upon its circumstance? Perhaps guppies use visual cues when olfaction and gustation cues are restricted or *vice versa*. The midas cichlid may be a good example of a fish which exhibits context-dependent use of sensory modality (Barnett 1982). In a Y-maze where only chemosensory responses could be demonstrated, midas cichlid fry choose adult females (not necessarily their own mother) over males and other stimuli presented to them (Barnett 1982). However, vision may also important in the discriminatory ability of cichlid fry to recognise each other (e.g. Hay 1978) and Barnett (1982) suggested that both sensory modalities are used by cichlid fry. Fry use vision during conditions of clear water so that they stay near the centre of the territory guarded by a parent and thus avoid predation. When water turbidity or light intensity is low and visual cues are therefore reduced chemosensory recognition is relied upon. For guppies in the wild it may be that visual cues are used during daylight hours while chemosensory recognition of school-mates is relied upon during periods of darkness, or during the rainy season when river floodwater is very turbid.

Data described in chapter 7 demonstrate that number of potential schooling partners is one factor which mediates schooling preferences for certain types of fish. Preference for familiar school-mates by guppies in the Upper Tunapuna River decreases as group size from which the fish were obtained increases (chapter 7), suggesting a possible role for individual recognition. The level of resolution of discrimination (species, group, or individual recognition) depends upon the information available about potential school-mates, which in turn may depend upon sensory modality. Brown & Smith (1994) suggest that group recognition may be achieved by shared group odour. Future work may investigate the possibility that additional information provided by visual cues allows individual recognition to be achieved. Alternatively, the level of resolution of discrimination between school-mates may not depend upon the sensory modality fish use to recognise each other, but on prevailing environmental factors such as predation risk and food availability. Perhaps individual recognition is only demonstrated by fish when the benefits outweigh the

costs incurred. The anti-predator (Chivers *et al.* 1995) and foraging (Dugatkin & Wilson 1992; Metcalfe & Thomson 1995) benefits to individual fish choosing familiar school-mates is now understood. Under certain conditions, these benefits may be further increased by preferential association with particular familiar individuals.

Kinship experiment

Contrary to expectations, juvenile guppies did not prefer kin as school-mates. Neither visual nor chemosensory stimuli provided information upon which the fish established preference for or avoidance of related conspecifics. Future work, however, should test the possibility that a combination of these cues might provide enough information for kin recognition to be achieved. Alternatively, it could be that levels of relatedness were not higher within broods than between broods, and that a prerequisite of the experimental protocol was therefore not fulfilled. This might have been the case if brood paternity was not wholly attributable to 1 male. Female guppies store sperm for long periods of time: Tunapuna and Tacarigua females are able to produce broods on average for 3 and 4.5 months respectively without receiving further matings (A. E. Magurran & B. H. Seghers pers. comm.). Some of the juveniles in this experiment may have been fathered by sperm from a mating which occurred previous to the isolation of females in bottles. However, Grove (1980) in Reynolds *et al.* (1993) has suggested that the greater proportion of a brood is fertilised by sperm from the most recent full copulation and as each brood must share maternally inherited genes, relatedness would almost certainly have been higher within than between broods in this experiment.

Multiple matings are commonplace for females in the wild. They can receive up to one sneaky mating attempt per minute (Magurran & Seghers 1994c). Although the proportion of a wild brood fathered by any one of many male partners has yet to be elucidated, it seems likely that relatedness within wild born broods will be lower than relatedness within the experimental broods. The experimental protocol described above has ensured maximum opportunity for broods to be related and therefore for the expression of preferences by guppies for kin. As a result, the schooling preferences of wild fish will not have been underestimated.

If a preference for related over unrelated conspecifics had been noted, the possibility that the juveniles were basing their schooling decisions on familiarity developed during the 28 days preceding birth rather than on the basis of kinship could not have been discounted. There is no evidence that pre-birth familiarity affected the schooling decisions of juveniles. It is suggested, therefore, that the recognition of familiar conspecifics noted in the first experiment of this chapter develops after birth. An experiment similar to that described in chapter 6 would need to be conducted in order to test this hypothesis. Perhaps guppies from the same brood become familiar with one another more quickly than guppies from different broods.

A recent study of a domestic guppy strain suggested that relatedness was a factor upon which guppies based schooling decisions (Warburton & Lees 1996). Although it seems that Warburton & Lee's data contradict those collected for descendants of wild fish in this investigation, closer inspection of their experimental protocol reveals that kin groups were raised together for 1 week between time of birth and trials. The authors suggest that preference for related conspecifics may in fact be partly attributable to preference for familiar conspecifics (Warburton & Lees 1996). The guppy is a fish where juveniles from the same brood have a good chance of remaining together until adulthood. Not only are guppies ovoviviparous (Thibault & Schultz 1978) and livebearing (Wourms 1981) - juveniles developing internally over a period of 1 month before birth - but juveniles are able to perform co-ordinated schooling behaviour immediately after birth (Magurran & Seghers 1990). Despite this, kinship does not seem to play an important role (if any) in the schooling decisions of juvenile guppies. It remains to be seen if the recognition abilities of guppies is facilitated by ontogenetic changes in sensory systems, for example (chapter 9).

Are wild guppy schools composed of related individuals? Although this study has shown that guppies do not prefer kin over unrelated schooling partners, this does not preclude the possibility that guppy schools are composed of related individuals, or that kin recognition may be a phenomenon only demonstrated by wild fish. Guppies have the opportunity to become familiar with kin and may preferentially associate with these individuals on the basis of familiarity alone. Indeed multilocus

fingerprinting techniques have revealed that wild guppy school members are more closely related than the average for the population (Magurran *et al.* 1995). Despite this, the level of relatedness within schools was low, in the range of 4th order relatives (Lynch 1988). See chapter 5 for a discussion of possible sex differences in schooling preferences and school fidelity which may account for this finding.

Previous to work described by Magurran *et al.* (1995) investigations of relatedness in fish focused on population differentiation and speciation. Ferguson & Noakes (1981) measured the genotypic structure of natural stocks of the common shiner, *Notropis cornutus*. The heterogeneous gene frequencies revealed evidence for restrictions on genetic variation of these stocks. Six groups of common shiner were caught during their breeding period. The groups were found to be neither random assemblages of individuals nor were they genetically isolated from the other groups. Thus Ferguson & Noakes (1981) suggested that schooling behaviour might well be associated with kinship, although data were not available to test this hypothesis directly.

More recently, Naish *et al.* (1993) collected schools of European minnow from the River Frome, Dorset, in order to test the possibility that relatedness was higher within than between schools. Not only were nuclear and mitochondrial DNA markers unable to indicate this relationship, but in fact levels of relatedness were found to be lower within than between schools. In future, the limitations which allozyme analyses have placed on studies of kinship may be lifted by techniques such as microsatellite analysis which allow finer resolution of DNA samples (Queller & Strassmann 1993). This will be especially important for studies of relatedness in fish due to the evolutionary implications of altruistic interactions within schools and reduced gene flow within populations.

CHAPTER 9

Conclusions and future directions

The aim of this thesis has been to investigate the individual decision making abilities of schooling fish in order to answer the question: do individuals choose particular school-mates, and if so, on what basis are these decisions made? The null hypothesis that schools are composed of random assortments of individual fish has been rejected. School membership and structure are profoundly affected by the cognitive abilities and partner choice decisions of fish. Field work carried out in Trinidad and Dorset, UK (on guppies, *Poecilia reticulata*, and European minnows, *Phoxinus phoxinus*, respectively) has demonstrated that familiarity does indeed influence choice of schooling partner. Individual guppies under laboratory conditions and in the wild recognise and prefer school-mates with whom they are familiar (chapters 3 and 5), and schools of minnows in semi-natural conditions are composed of a significant proportion of individuals which are familiar to one another (chapter 4, Griffiths 1997). Furthermore, the effect of group size on these partner choice decisions suggests that individual recognition may be possible (chapter 7, Griffiths & Magurran 1997b). Schools are by no means composed of a random assortment of individuals. Indeed, school structure and membership are profoundly affected by the remarkable discriminatory abilities of individual fish.

This chapter will discuss how the discriminatory abilities of fish revealed by this thesis will be important in the development of future research in schooling behaviour. Three issues of particular interest will be considered. First, how the individual decision making abilities of schooling fish, although now understood to be remarkable, may yet prove to be even more extraordinary. Second, the implications which individual partner choice preferences have for the movement and membership of schools in the wild. Finally, consideration will be given to the role of kinship in schooling decisions and its potential effect upon gene flow and speciation.

9.1 Individual decision making abilities of schooling fish

How do the decisions of individual fish produce the patterns of schooling behaviour observed in the wild? Data presented in this thesis demonstrate that fish prefer to school with certain individuals and previous experience, termed familiarity, appears to play an important role in choice of schooling partner. Understanding the role of familiarity in schooling decisions is important because it may reveal something of how schooling evolved. Non-random associations between individuals may allow members of a group to behave co-operatively via a strategy of reciprocity for example (Dugatkin 1997). The role which familiarity may play in the co-operative alliances between fish, e.g. during tit-for-tat behaviour is discussed below. Furthermore, if there are advantages to recognising and preferentially associating with familiar conspecifics in addition to the benefits of schooling *per se* (see chapter 1) then this might be another factor contributing to the evolution of schooling. Indeed it is known that schools composed of fish familiar with one another may gain foraging (Metcalfe & Thomson 1995) and anti-predator benefits (Chivers *et al.* 1995).

(a) Motivation

What other factors might influence school structure and membership? Perhaps fish make moment by moment partner choice decisions on the basis of motivation. Do fish prefer to school with familiar conspecifics when they are hungry or under risk of predatory threat? If this were true each individual would be able to choose from amongst school-mates of known competitive ability or proficiency of anti-predator manoeuvres. Foraging with individuals of poorer competitive ability is presumably advantageous (Metcalfe & Thomson 1995), and even 'losers' in these relationships may benefit by avoiding costly contests over resources (e.g. Pagel & Dawkins 1997). Chapter 4 shows that River Frome minnows do not increase their schooling preference for familiar conspecifics with increased risk of predation (Griffiths 1997). However, it remains to be seen if hunger level affects schooling preferences. Indeed fish may trade-off the advantages of choosing familiar school-mates against the anti-predator and foraging costs and benefits of

schooling. One way in which this might be envisaged is through the positional preferences demonstrated by fish. Occupying particular positions within a school may carry different costs and benefits (Krause 1993c). For example, the feeding rate which can be achieved by roach, *Rutilus rutilus*, occupying positions at the front of a school is higher than for roach in other positions (Krause 1993b). Similarly, frightened fish (exposed to Schreckstoff) move to central school positions relative to fish habituated to the presence of Schreckstoff (Krause 1993a). It seems therefore that individuals at risk of attack gain pre-attack benefits (Smith 1997a) by adjusting their position within a school. While the tendency to move to central positions when predatory risk is high is known to be affected by factors such as hunger level (Krause 1993b) and parasitism (Krause & Tegeder 1994). It is possible that familiarity may also affect such behavioural responses. It is hypothesised that members of schools of familiar fish may be able to delay moving to central and safer positions for longer than individuals from schools of unfamiliar fish because of their more efficient anti-predator repertoire (Chivers *et al.* 1995). As a result they may be able to minimise the cost of moving position i.e. the cost incurred by abandoning their current activities. In future it would be interesting to investigate the interaction between familiarity and motivation in more detail.

(b) Ecological parameters

It would also be interesting to determine whether individual partner choice preferences for familiar, natural school-mates are influenced by ecological parameters. The rivers of Trinidad would be a good place in which to test this question as guppies from different river populations are exposed to different levels of predation pressure and food availability (Haskins *et al.* 1961; Seghers 1973; 1974; Liley & Seghers 1975; Fraser & Gilliam 1987). It is predicted that the degree of preference for familiar individuals in a low predation regime will be magnified in localities where there are many predators. The extent of this preference is expected to be greater in habitats where there are many predators because the potential benefit of associating with familiar school-mates is larger for fish under high risk of predation (Chivers *et al.* 1995). On the other hand, in high predation sites, it may simply be less risky to school with any conspecific rather than leave the safety of a school in search of familiar individuals. Data presented in chapter 7 demonstrate that the expression of familiarity is constrained by group size. Preferences are

strong when fish originate from small groups but decline as group size increases (Griffiths & Magurran 1997b). The effect of group size upon partner choice decisions suggests that guppies may be employing individual recognition - an ability which may profoundly influence school structure. Paradoxically, the factor which confers anti-predator protection to schooling fish, that is similarity in appearance, is also the factor which makes individual recognition difficult. Thus, the greater schooling tendency and larger school size of guppies from high compared to low predation populations (Seghers 1974a) may also effect a more complex interaction between school size and predation regime.

(c) Co-operation

The ability of fish to repeatedly recognise particular conspecifics (Milinski *et al.* 1990a) and the association of familiar fish within groups suggests that naturally occurring schools would be likely places in which the formation of co-operative alliances between individuals might be found. Recognition and familiarity (which may result in non-random interactions, Hamilton 1964) are pre-requisites to the evolution of co-operation and preferential association with familiar individuals may therefore allow a strategy of reciprocal altruism to be forged (Trivers 1971). Circumstances under which co-operation has been observed include predator inspection behaviour (Milinski 1987; Dugatkin 1988) where two or more individuals approach a potential predator in order to confirm its identity and assess its motivation to attack (Pitcher *et al.* 1986a; Magurran & Pitcher 1987; Pitcher 1992), and group foraging behaviour (Major 1978; Mittlebach 1984; Dugatkin & Wilson 1992). Threespine sticklebacks, *Gasterosteus aculeatus*, are known to choose inspection partners with whom they have successfully performed predator inspection behaviour in the past (Milinski *et al.* 1990b) and it seems likely that association with familiar school-mates whose trustworthiness is known from previous interactions would allow this strategy to become established quickly and more efficiently.

In chapter 5, sex differences in schooling preferences were found for wild guppies. Only female guppies school preferentially with familiar, natural school-mates while males do not make this distinction. It seems likely that in the wild males move opportunistically between schools in order to maximise their chances of mating. However, females must associate with one another for fairly

long periods of time in order to become familiar and may therefore form the core of wild schools. It is predicted that future work may demonstrate that co-operative alliances, such as observed during tit-for-tat behaviour, are formed significantly more often between females than between males and females. Indeed, because the acquisition of familiarity occurs gradually (chapter 6, Griffiths & Magurran 1997a), males are unlikely to co-operate with each other simply because they do not school together for long enough periods of time to allow familiarity to develop. An exception to this may be during times of flood when water and turbidity levels are high. During this time males have been observed to swim together downstream in large schools (J. A. Endler pers. comm.), and it is possible that the males remain faithful to these schools for long enough to become familiar in the same way as females. Alternatively, it may be that once familiarity has developed, individuals may be able to recognise their school-mates even after long periods of separation.

For how long are fish able to remember their familiar school-mates? It is known that preference for familiar conspecifics is established gradually (Griffiths & Magurran 1997a). Furthermore, some fish retain their preference for former school-mates even if they have been separated for periods of 10 days (threespine sticklebacks, Van Havre & FitzGerald 1988) or two months (fathead minnows, *Pimephales promelas*, Chivers *et al.* 1995). It would be interesting to undertake an experiment to test for how long after being separated from natural school-mates individuals are still able to recognise these 'familiar' fish, because the formation of long term alliances has the potential to profoundly affect school structure. Future work might test the precise time scale over which these preferences are maintained.

(d) Response to Schreckstoff

The putative alarm pheromone, Schreckstoff, is a substance found in the club cells of ostariophysan fish. Club cells are embedded in the epidermis and are non-secretory (Pfeiffer 1977; Smith 1977; Smith 1992). Schreckstoff is therefore only released upon mechanical damage to the skin of these fish (von Frisch 1941; Smith 1992; Hara 1993), such as incurred during predator attack. While early explanations for the evolution of Schreckstoff relied upon group selectionist theories (fish producing Schreckstoff so as to warn other group members of

impending danger), it has been more difficult recently to understand the benefits to individual fish of this activity. The problem with accepting Schreckstoff's alarm signal function is that current evidence does not suggest that school members are related to one another (Ferguson & Noakes 1981; Avise & Shapiro 1986; Dowling & Moore 1986; Naish *et al.* 1993). and therefore kin selection is unlikely to explain the evolution of Schreckstoff as an alarm pheromone (Williams 1964; Williams 1992). Moreover, the function of Schreckstoff under natural conditions is currently under debate (Magurran *et al.* 1996; Smith 1997b). In a study by Irving & Magurran (1997) the importance of naturalness in laboratory experiments was investigated by measuring the fright responses of European minnows following an exposure to identical initial concentration of Schreckstoff. With increasing naturalness of the experimental conditions (from sealed aquaria with no through flow of water to the near-natural conditions of a fluvarium through which the entire flow of the River Frome Mill Stream passed) the severity of the alarm response decreased (Irving & Magurran 1997). More recent work by Magurran *et al.* (1996) has cast further doubt on the alarm signal function of Schreckstoff. Wild minnows in the River Frome failed to modify their behaviour or undertake area avoidance when conspecific skin extract was released nearby. It seems, therefore, that the current function of Schreckstoff may not be as an alarm signal, indeed the nature of the signal itself is unclear.

Although club cells are the source of Schreckstoff (Smith 1992), it seems that even fish which do not possess these cells demonstrate a reaction to conspecific skin extract. For example, guppies from the Quare River in Trinidad responded to the controlled release of conspecific skin extract with classic fright responses such as area avoidance, decrease in number of predator inspection visits, increase in schooling and frequency of 'dashing' (J.-G. J. Godin & G. E. Brown, pers. comm.). Such alarm responses were noted for guppies exposed to the release of conspecific skin extract under laboratory conditions, in a field trapping experiment, and when presented with a realistic predator model in the wild. One reason which might explain this peculiarity is that alarm signalling is a secondary function of Schreckstoff or similar skin extract. An alternative to the alarm signal hypothesis is the anti-pathogen hypothesis put forward by Smith (1982), Hugie (1987) and Irving (1996), although these hypotheses may not be mutually

exclusive. Perhaps club cells and Schreckstoff originally evolved to fight pathogenic attack (Smith 1982) and Schreckstoff release is now used as a reliable indicator of predator-induced injury.

Although the function of Schreckstoff remains unclear its release has often been found to elicit anti-predator behaviour. Perhaps familiarity and co-operation play a role in the interaction of fish during the release of a chemical odourant such as Schreckstoff in the same way as during the inspection of a visually presented predator. Do fish preferentially associate with familiar school-mates when conspecific skin extract is released? Behavioural responses to Schreckstoff are known to include reduced feeding rates (Magurran 1989a), increased school cohesion (Heczko & Seghers 1981), increased shelter use (Mathis *et al.* 1993) and area avoidance (Hemmings 1966; Mathis & Smith 1992). These behaviours potentially reduce the risk of predation and a situation can be envisaged where choice of familiar schooling partner during the performance of these responses could result in an increased anti-predator advantage to these discriminating individuals. Are groups of familiar fish able to detect Schreckstoff and perform anti-predator tactics earlier or more efficiently compared to groups of fish which are unfamiliar to one another? The role which familiarity may play in the response of schooling fish to the release of Schreckstoff has yet to be investigated although it is one further way in which the recognition abilities and partner choice decisions of fish may affect school membership in the wild.

9.2 Movement of schools in the wild

The study of partner choice decisions of individual fish is a good way in which insights into patterns of school structure can be gained. Most initial work has focused on partner choice preferences in situations where the potential for adaptive costs and benefits are great, such as in the contexts of foraging or anti-predator behaviour (see Dugatkin & Wilson 1993). Recognition of school-mates on the basis of familiarity alone takes time to develop (chapter 6, Griffiths & Magurran 1997a). Despite this, the benefits of condition-independent recognition (chapter 6) may in fact have profound effects upon school structure and movement in the wild. Because it takes time for fish to get to know one

another, individuals must therefore stick together for a relatively long time in the wild in order to subsequently be capable of recognising familiar school-members (Griffiths & Magurran 1997a). The preferential association of familiar fish within naturally occurring schools might lead to the expectation that levels of school fidelity are relatively high. However, there have been few attempts to investigate the schooling preferences of wild fish and it is still not known how school membership and movement varies over space and time (Helfman 1984).

Future work might aim to recapture schools of fish over varying periods of time and in doing so gain information about temporal variation in school membership and size. By re-sampling schools of fish over many km, information about spatial variation in school fidelity would also be gained. Information of this kind regarding school membership may be especially important to understanding the spatial position of fish schools in the sea and might therefore have implications for fisheries management policies (Fréon *et al.* 1989; Irving 1996; Fréon & Misund 1998). Data from one of the most successful mark-recapture studies conducted to date are reported by Hilborn (1991). He showed that levels of school fidelity are not high for skipjack tuna, *Katsiwonus pelamis*, and that between 16-63% of individuals leave a school each day to join other schools. The data set from which these conclusions were been drawn described tagging of 150,000 skipjack tuna from 5,000 schools in the western tropical Pacific between 1977 and 1980 (Kearney 1983). It is worth noting, however, that of the 150,000 individuals tagged only 6,000 returns (4%) were found, highlighting the difficulties faced by mark-recapture studies. Although schooling fish are the foundation of the fisheries and fishing industries it is this schooling behaviour which limits both the application and accuracy of stock assessment methods (Ulltang 1980; Fréon *et al.* 1989; Aglen 1994). Despite the inherent difficulties of mark-recapture surveys data do exist to suggest that familiarity may be important in predicting the schooling patterns of marine species as well as freshwater fish (Lester *et al.* 1985; Bayliff 1988). What will be required of future work is a comprehensive sampling effort for whole fish populations.

In another summary of skipjack tuna tagging and recapture data, Bayliff (1988) suggested that the greater part of exchange of individuals between schools took place within 3-5 months after tagging. This would be enough time for individuals to become familiar with one another and therefore gain the advantages of schooling with familiar conspecifics. What has not been discounted is the possibility that some fish form very cohesive cores (sub-units) within schools (Sharp 1981). Small groups of individual fish associating together within a sub-unit would be predicted to become familiar with one another within a relatively short length of time, and foraging and anti-predator benefits would therefore be expected to be afforded to these discriminatory fish.

Evidence of the existence of sub-groups within single species schools is provided by Partridge (1981) who observed schools of saithe containing more than 10 individuals. Schools of herring, *Clupea harengus*, (Pitcher & Partridge 1979) and minnows (Pitcher 1973a) have also been observed to contain sub-groups where particular individuals associated more closely with one another than would be expected if the school-members were distributed at random. Spatial segregation between species has also been described (Hobson 1963; Hobson 1968; Parrish 1989). Parrish (1989) observed the aggregation of morphologically and ecologically similar fish within single schools. These species were arranged in a typically tri-layered pattern so that the surface layer was usually occupied by small-sized atherinids, *Allanetta harringtonensis*, the middle layer by juveniles and adults of a clupeid, *Jenkensia lamprotaenia*, as well as juveniles of an engraulid, *Anchoa choerostoma*, and another clupeid, *Harengula humeralis*, while the lowest layer was occupied by larger sized adult *A. choerostoma* and *H. humeralis*. Within such mixed-species layers Parrish (1989) noted that small discrete single-species schools would often form. It seems quite likely that fishermen's operational definition of a school may in fact include fish from many functionally distinct schools.

9.3 The role of kinship in schooling decisions

(a) Interactions between kinship and familiarity

The schooling decisions and partner choice preferences of individual fish are known to be affected by familiarity. However, familiarity may have arisen either because individuals recognise one another on the basis of past interactions, or, alternatively, because they are related, and therefore employing kin recognition. Many species of fish preferentially associate with kin (table 1) and even species which do not prefer kin under laboratory conditions may form schools composed of related conspecifics. For example, although the juvenile guppies described in chapter 8 were found to prefer familiar conspecifics, unfamiliar kin were not preferred to unfamiliar unrelated individuals. Yet, this does not preclude the possibility that both kinship and familiarity play a part in the schooling decisions of individuals in the wild. It remains to be seen if schools are composed of familiar or related individuals or both. Furthermore, the interaction between kinship and familiarity may be mediated by ontogeny. Perhaps kin recognition is only employed by mature but not juvenile guppies (chapter 8), in which case only schools of sexually mature individuals would be likely to be composed of kin. This possibility is certainly worthy of further investigation.

Familiarity may develop between related individuals as a result of a number of factors. Broods of juvenile siblings which swim, or are swept together, into a nursery area of river habitat (marginal areas of low current speed, often with vegetation, Mills 1991) may have the opportunity to become familiar with one another and associate with one another subsequently. Mann & Mills (1986) found that juvenile dace, *Leuciscus leuciscus*, collected at spawning sites in the River Frome immediately after hatching could only hold station at about 17 mm s^{-1} and as a result were washed into marginal habitats where water current was < 20 mm s^{-1}. Common rearing of juveniles as a result of mouth brooding (e.g. African cichlids, Fryer & Iles 1972), nest defence (e.g. threespine sticklebacks, Wootton 1984) or internal fertilisation (as is the case for guppies) may also facilitate the development of familiarity between siblings. It seems that species with these life history characteristics are the most likely to form schools composed of related

individuals and although the most parsimonious explanation for relatedness among school members may not be kin recognition (Grafen 1990), initial efforts to find evidence of kinship within schools should be focused on these fish.

(b) Levels of relatedness within wild schools

The study of relatedness in fish schools is important for two reasons. First, if individual fish are able to increase their inclusive fitness by behaving altruistically toward related conspecifics, then kin selection (Hamilton 1964) may be a part of the mechanism by which schooling evolved. Second, the association of relatives within schools could reduce gene flow through the metapopulation. Evidence that wild schools are composed of related individuals is sparse and equivocal (see chapter 8, Ferguson & Noakes 1981; Avise & Shapiro 1986; Dowling & Moore 1986; Naish *et al.* 1993) but the low level of resolution which techniques such as allozyme analysis can reach has limited a thorough investigation of this question. Future work is planned which aims to employ microsatellite analysis of schools of the guppies collected from the wild in December 1996 and whose schooling preferences are recorded in chapter 5. Thus any evidence for kin-grouping will reflect the schooling decisions of these wild fish. In this way the question of whether schools are composed of familiar or related individuals or both can begin to be addressed.

(c) The role of gender in genetic structuring of schools

Data presented in chapter 5 revealed interesting behavioural differences between the sexes for guppies. Females preferred schooling with females from the school they belong to in the wild. Males, however, did not prefer their natural, same-sex school-mates to unfamiliar fish. This suggests that males trade the foraging and anti-predator benefits of schooling and of associating with familiar conspecifics against the mating advantages of moving between schools of females. Mark recapture studies in the wild may reveal mobility levels to be higher among males than among females. Males have a much higher potential reproductive output, although to realise this, they must be attractive to many partners, or be able to outcompete male rivals and undermine female choice (Clutton-Brock & Parker 1995). Females usually invest most in their offspring (Bateman 1948) and tend to be the choosy sex. It is perhaps this sexual asymmetry in mating costs (Trivers 1972; Parker

1983) which has lead the two sexes to establish different partner choice preferences during schooling.

The observation of a sex difference in schooling partner preference leads to the prediction that levels of relatedness will be higher among female members of wild guppy schools than among males. Although a previous investigation of kinship amongst members of guppy schools established that levels of relatedness were low, data were not analysed with regard to gender (Magurran *et al.* 1995). It is hoped that future work will discover whether sex differences in schooling preferences are underlain by sex differences in relatedness within schools. This may be the case not only for guppies but perhaps also for other fish species which are sexually dimorphic or have asymmetrical mating costs.

(d) Gene flow and speciation

As mentioned above, the study of relatedness in fish schools is important because the association of relatives within schools could reduce gene flow through the metapopulation. The guppy is a classic example of evolution in action (Magurran 1996), and much is already understood about its behaviour. It is therefore an ideal species in which to study the role of kinship in schools. Investigations of relatedness and mobility within and between guppy schools may reveal much about how speciation is facilitated by evolution.

On the basis of the evidence presented in chapter 5 for female guppies it might be expected that levels of gene flow between schools would be quite low. Not only must females remain school-mates for relatively long periods of time in order to become familiar (chapter 6, Griffiths & Magurran 1997a), but female guppies are very choosy amongst males. Houde (1997) reviews the evidence for female preference for males with most and brightest carotenoid (red-orange) colour patterns (e.g. Kodric-Brown 1985). These factors suggest that gene flow is restricted and therefore that rates of speciation between guppy populations should be correspondingly high. Intriguingly, this is not the case. The fish in the Caroni and Oropuche drainages (see chapter 2) have been separated for a period of 330,000 years (estimated from mtDNA, Fajen & Breden 1992) - 500,000 years (estimated from allozymes, Carvalho *et al.* 1991). Furthermore, the genetic distinctness

of these drainages and also of populations within each drainage are marked (see Magurran *et al.* 1995, for an overview). Despite this, speciation has not occurred (Magurran *et al.* 1995).

One factor which may enhance gene flow and thus slow down the rate of speciation is the sneaky mating behaviour (gonopodial thrusting) of male guppies (see chapter 2). Recently it was found that males which perform the highest rates of sneaky mating also contain the greatest number of sperm (Matthews *et al.* 1997) and thus have the capacity to significantly undermine female choice. Another way in which gene flow may be enhanced is through high levels of male mobility (as suggested in chapter 5 and section 9.3). If males move between schools in search of mating opportunities then the potential for gene flow restriction as a result of female discriminatory abilities (both schooling partner preference and mate choice) will be undermined. It is predicted that future work may reveal sex differences in mobility either directly through mark-recapture studies or indirectly by measuring differences in rate of maternal and paternal DNA transfer through a metapopulation.

To conclude, this chapter has outlined a number of interesting avenues of research which warrant further investigation, as well as some ideas for new directions. It will be particularly important in future work on schooling behaviour to successfully integrate laboratory investigations with field observations, and the following issues are suggested as important and interesting themes to persue and develop.

- Individual decision making by members of schools especially the role of ecological parameters such as predation risk and food availability.
- School fidelity and movement of schools in the wild.
- The genetic structuring of fish schools and metapopulations, especially with regard to sex differences in behaviour patterns.

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Familiarity in schooling fish: how long does it take to acquire?

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Abstract. Previous work has demonstrated that fish prefer to school with familiar individuals. In this study schooling preferences for familiar female guppies, *Poecilia reticulata*, developed gradually over 12 days, but once established were maintained. This contrasts with condition-dependent recognition in which fish rapidly learn to discriminate between conspecifics on the basis of obvious morphological differences such as body size.

In recent years it has become evident that fish can discriminate between conspecifics and that this ability plays an important role in individual decision making (Dugatkin & Sih 1995). For example, European minnows, *Phoxinus phoxinus*, prefer to associate with poor competitors since they presumably gain foraging advantages by doing so (Metcalfe & Thomson 1995), while three-spined sticklebacks, *Gasterosteus aculeatus* (Milinski et al. 1990a, b) and guppies, *Poecilia reticulata* (Dugatkin & Alfieri 1991) remember the outcome of past encounters when selecting partners for predator inspection.

Schooling behaviour is a highly effective strategy against predators (Magurran 1990); larger schools are not only more vigilant (Magurran et al. 1985), but also less vulnerable to attack by pike, *Esox lucius*, and other predators including squid, *Loligo vulgaris*, cuttlefish, *Sepia officinalis*, and perch, *Perca fluviatilis* (Neill & Cullen 1974). Although it is recognized that individual fish that differ in appearance or behaviour may be at greater risk than their schoolmates as a consequence of the oddity effect (Landeau & Terborgh 1986), scant attention has been paid to how school membership influences the effectiveness of schooling as an anti-predator device. For instance, there has been little consideration of the way in which individual recognition might enhance the anti-predator benefits of schooling. However, Chivers et al. (1995) have recently demonstrated that the behaviour of fathead minows, *Pimephales promelas*, varies depending upon whether the schools to which they belong are composed of familiar or unfamiliar individuals. Chivers et al. found that 'familiar' schools are more cohesive and more frequently adopt behaviours, such as predator inspection, that bestow greater protection on school members. This result, along with other studies that have demonstrated schooling preferences for familiar fish (Van Havre & Fitzgerald 1988; Magurran et al. 1994; Chivers et al. 1995), implies that individual recognition is a neglected aspect of schooling dynamics.

Individual recognition may be beneficial but how is it achieved? The oddity effect selects against individual variation in appearance and one of the most striking features of natural schools of fish is the degree to which the school members resemble one another. Thus the very factor that confers protection, similarity in appearance, is also one that makes individual recognition more challenging. Although there is evidence that fish can easily distinguish between conspecifics on the basis of obvious morphological differences such as body size (Ranta et al. 1992), recognition based on familiarity may be slower to develop. Previous investigations of the role of familiarity in decision making have examined groups of fish that were kept together for long periods. Magurran et al. (1994) looked at schooling preferences for familiar fish in groups of up to 15 guppies that have been together for 2 months. Brown & Smith (1994) and Chivers et al. (1995) collected naturally occurring schools of fathead minnows from the wild and then

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maintained the schools in separate aquaria until they were ready to be tested. Metcalfe & Thomson (1995) examined the schooling preferences of European minnows kept in groups of seven for between 12 and 20 days (N. B. Metcalfe, personal communication). Dugatkin & Wilson (1992) found that bluegill sunfish, *Lepomis machrochirus*, that had been in groups of six for more than 3 months preferred to associate with familiar individuals. How long does it take for fish to begin to treat 'familiar' individuals as preferential schooling partners? To answer this question we examined the schooling preferences of female guppies.

Female guppies are cryptic and uniform in appearance, unlike their male counterparts which are brightly coloured and so polymorphic that no two individuals resemble one another (Magurran et al. 1995). Female guppies also have a higher schooling tendency than males and are more likely to adopt anti-predator tactics in threatening situations (Magurran & Seghers 1994b). The population of guppies that we used in this experiment, the Lower Tacarigua, is derived from a site where there are many predators. As might be expected, females from this population school strongly (Magurran & Seghers 1994a) and have the potential to benefit from the 'familiarity' benefits that Chivers et al. (1995) identified.

METHODS

The individuals used in this experiment were normally held as large breeding stocks in the laboratory, and were descendants of guppies collected from the wild in Trinidad 18 months previously. We removed 36 females of as similar a size as possible (mean total length $\pm$ SE$=31.8 \pm 0.58$ mm) from three stock tanks and placed them in an aquarium together for 4 days to allow complete mixing before separating them into six groups. This ensured that the fish were equally unfamiliar at the beginning of the experiment. Each group of six fish was allocated its own aquarium ($45 \times 32 \times 32$ cm deep) so that the groups were isolated visually and olfactorily from one another. Each tank contained a water filter, air stone and clump of Java moss, *Vesicularis dubyana*. The fish were fed daily with Tetramin and kept at 25°C on a 12:12 h light:dark regime. Fish that shared a tank were designated 'familiar' fish whereas fish in different tanks were known as 'unfamiliar' fish.

There were two parts to the experiment. First, over a month we repeatedly tested individual guppies for their schooling preferences for familiar and unfamiliar fish. Second, to investigate the possible effect of repeated exposure to the experimental procedure on the acquisition of familiarity, we selected a further 36 females (mean total length $\pm$ SE$=32.4 \pm 0.62$ mm). In this control experiment individuals were only given the choice between schooling with familiar and unfamiliar fish once, that is 12 days after they had been separated into six groups.

We measured individual schooling tendency using a similar procedure to that adopted by Magurran et al. (1994) after Keenleyside (1955). Two clear plastic 1-litre bottles (22 cm high) were positioned 6 cm from either end of a test tank ($90 \times 32 \times 32$ cm filled with water to 20 cm). The bottles were punctured to allow visual and olfactory communication. Before a trial, we placed four females from each of two randomly chosen groups in each of two separate bottles. We intended the test guppy to have the opportunity either to school with the familiar fish (from its own tank) or unfamiliar fish (from another tank) or to remain solitary. After 15-min settling time, we released the test fish into the centre of the test aquarium. The trials were conducted on days 1, 2, 4, 8, 12, 15, 19, 22 and 26, as well as on day 12 of the control experiment.

We measured schooling behaviour by recording how long the test female spent within 10 cm of each bottle. Trials lasted 15 min. Two fish per group were tested each day. We randomized the order of the trials, and no fish was tested more than once each day. The position of the bottles was also randomized. We measured the total length of the fish in each group at the end of the experiment in order to confirm that fish size did not vary significantly between tanks.

RESULTS

All proportion data were arcsine transformed (Sokal & Rohlf 1995) before analysis. Since there was no significant difference in the percentage of time that the two test females from each group spent schooling (one-way ANOVA: $F_{1,214}=0.32$, $P=0.57$), data from both females were included in the analyses of schooling preference. The statistical analyses used was a repeated measures

Table I. Repeated measures ANOVA of guppy schooling behaviour, measured as the percentage of time spent with stimulus fish in choice tests

Source	*df*	Mean square	*F*	*P*
Familiarity	1	4821.8	9.43	0.003
Group	5	295.4	0.58	0.717
Familiarity*group	5	1271.0	2.49	0.036
Day (group)	48	76.4	0.15	1.000
Familiarity*day (group)	48	950.7	1.86	0.004
Error	108	511.1		

The factors are familiarity, day of testing and group. Day of testing is nested within group. Data were arcsine transformed.

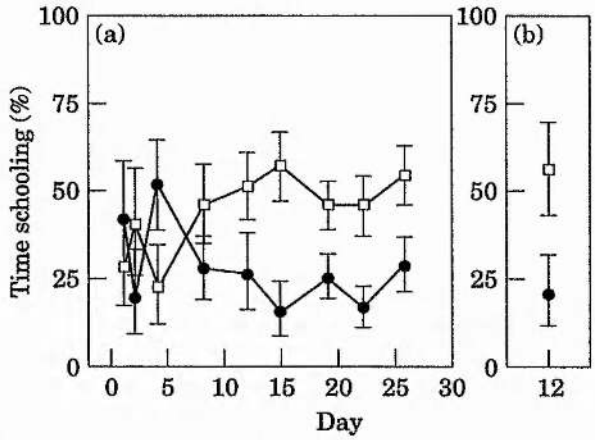

Figure 1. The percentage of time a female guppy devoted to schooling with familiar (□) or unfamiliar (●) guppies: (a) when this choice was presented repeatedly over 1 month; (b) after 12 days during which the test fish were not exposed to trial conditions. In all cases mean percentage time schooling ± SE (back transformed from arcsine percentage time data) is indicated (N=12 fish tested per day).

ANOVA in which day of testing was nested within group. Overall, female guppies preferred to school with familiar rather than unfamiliar conspecifics (Table I). The significant interaction between group and familiarity was due to the different rates at which groups developed preferences for familiar fish. This, however, did not change the overall preference for schooling with familiar conspecifics. The day of testing did not affect the proportion of time spent schooling. There was a significant interaction between familiarity and day indicating that a preference for familiar fish developed with time (Fig. 1a; Table I).

Day 12 was chosen as a suitable time for the control test as this seemed to be the critical point in the development of familiarity. After this time female guppies maintained a preference for familiar rather than unfamiliar schoolmates (Fig. 1a). Once again, there was no significant difference in the proportion of time that the two test fish in a group spent schooling (one-way ANOVA: $F_{1,22}$=0.00, P=0.995) and the results were pooled for the analysis of schooling preference. On day 12 of the control, female guppies with no previous experience of the experimental set-up or procedure preferred schooling with familiar schoolmates (one-way ANOVA: $F_{1,12}$=5.10, P<0.05; Fig. 1b). Interaction effects were not significant. The mean percentage of time spent schooling with familiar fish in this control (56.4%) was comparable to the value obtained on day 12 in the initial test (51.2%). This shows that the acquisition of familiarity by day 12 was not a consequence of repeated exposure to the experimental procedure.

The total length of the guppies did not vary significantly between groups in either the first experiment ($F_{5,31}$=1.26, P=0.307) or the control experiment ($F_{5,31}$=0.26, P=0.932) demonstrating that individuals were not choosing schoolmates on the basis of obvious morphological differences.

DISCUSSION

In this experiment female guppies, which had been living in groups of six, began to school preferentially with their tank-mates after a period of about 12 days. Once established, this predisposition to associate with familiar individuals persisted. Females continued to school with their tank-mates for the duration of the experiment, that is for a further 18 days. There is evidence that preferences can be maintained over longer time scales. Brown & Smith (1994) found that fathead minnows retained their preference for former schoolmates, even if they had been separated for 2 months or longer.

Our results indicate that recognition of familiar individuals takes some time to develop. This is not surprising given that female guppies are uniform and cryptic in appearance and that the individuals in our tests were size-matched and randomly allocated to the tanks. Yet there is evidence that fish may discriminate between particular individuals over a much shorter time frame. Milinski

et al. (1990a) investigated the predator inspection behaviour of sticklebacks that had been kept in groups of four for a 'few' hours. They found that in all cases at least some of the individual fish had partners with whom they preferentially inspected the predator. In a separate experiment, Milinski et al. (1990b) showed that sticklebacks could remember the better of two inspectors after seeing each of them inspect just four times. Dugatkin & Alfieri (1991) similarly found that guppies preferred to associate with the better of two inspectors. Three fish were placed in parallel channels and had 1 min in which to settle and a further 2.5 min in which to inspect a predator. Immediately afterwards the central fish was given a choice test in which it had the opportunity of associating with either of its erstwhile inspection partners. In 80% of trials there was a preference for the individual that had spent most time close to the predator. This level of discrimination persisted even if partner preference was tested 4 h after the inspection test, rather than at once. These tests reveal that individual fish can readily distinguish between conspecifics but do not prove that this discrimination was based on familiarity. It may be that the selection of partners during predator inspection is a form of condition-dependent recognition. Although Milinski et al. (1990b) suggested that the quickly established preference they saw was evidence for individual recognition, they could not exclude the possibility that the test stickleback had learnt to recognize the position of the better inspector rather than its actual identity. Support for the idea that fish assess their inspection partners on the basis of condition comes from an investigation by Külling & Milinski (1992), who showed that sticklebacks preferred to inspect in the company of larger individuals. Large individuals are preferred by the predator and hence more likely to distract the predator's attention from the smaller partner. It is also now well established that individual fish take the size of potential schooling partners into account when deciding with whom to associate (Ranta et al. 1992). Furthermore, it is possible that the hierarchical dominance of certain individuals over others may play a role in the schooling decisions of fish. Senar et al. (1990) showed that the social structure of siskin, *Carduelis spinus*, flocks are stable and that individual siskins joining a flock of unfamiliar birds will be subordinate to these existing flock members. The possibility that fish prefer not to school with unfamiliar conspecifics in order to avoid subordination cannot be discounted.

Since familiarity, as opposed to condition-dependent recognition, takes a number of days to develop we might expect fish to associate with particular individuals for protracted periods in the wild. There have been few attempts to investigate the schooling preferences of wild fish (see Helfman 1984 for a notable exception) but the subject is clearly ripe for further investigation.

Most studies to date have tested for schooling preferences between small groups of individuals and it may be that familiarity takes longer to develop, or is harder to achieve, in situations where there are more potential partners. However, Brown & Smith (1994) have demonstrated that olfaction plays a major role in the discrimination of familiar schoolmates. Indeed, they showed that fathead minnows can distinguish familiar individuals on the basis of chemosensory cues alone. The use of olfactory cues means that fish could base their schooling decisions on the odour of their habitual schoolmates and, so long as there is a shared group odour, the recognition of familiar individuals need not be constrained by group size. None the less, the existing evidence for individual recognition within schools (Dugatkin & Wilson 1992; Metcalfe & Thomson 1995) implies that group size may play an important role in the acquisition of familiarity. Here too, there is a strong case for further research.

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Schooling preferences for familiar fish vary with group size in a wild guppy population

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SUMMARY

The ability of fish to recognize and preferentially associate with familiar conspecifics has been well documented in a series of laboratory experiments. In this paper we investigate the schooling preferences of wild female guppies, *Poecilia reticulata*, in the Upper Tunapuna River in Trinidad and confirm that they do indeed prefer to associate with familiar individuals. The guppies in this river occur in a series of pools that become isolated during the dry season. These fish interact solely with other individuals in their pool for periods of several months at a time and thus have ample opportunity to become accustomed to one another. Our study also reveals that the tendency of female guppies to school with familiar fish declines as the group size in which they naturally live increases. Preferences are strong when there are small numbers of females in a pool, but diminish thereafter. This indicates that the expression of familiarity is constrained by group size. The basis of recognition and the consequences of schooling preferences for familiar individuals are discussed.

1. INTRODUCTION

There are many advantages of living in groups (e.g., Hamilton 1971; Wittenberger & Hunt 1985; Krebs & Davies 1993). In fish, the antipredator (Neill & Cullen 1974; Magurran 1990*a*; Pitcher & Parrish 1993) and foraging (Pitcher *et al.* 1982; Clark & Mangel 1984; Krause 1993) benefits of group-living have been particularly well documented. It has recently become clear that individual members of groups can enhance their fitness further if they associate with familiar rather than unfamiliar conspecifics. This effect was highlighted by Chivers *et al.* (1995) who discovered that schools of familiar fathead minnows, *Pimephales promelas*, were more cohesive and increased their cooperative antipredator behaviour compared with schools comprised of unfamiliar individuals. An overview of recent studies giving evidence for partner choice by fish in the contexts of antipredator and foraging behaviour, as well as in kin selection is presented by Dugatkin & Wilson (1993), and further examples from many different taxa are described by Dugatkin & Sih (1995).

Recognition of familiar individuals may be beneficial, but under what conditions can it be achieved? The ability to discriminate familiar from unfamiliar conspecifics has been demonstrated in a wide variety of freshwater fish species (including *Lepomis macrochirus*, Brown & Colgan 1986; *Gasterosteus aculeatus*, Van Havre & FitzGerald 1988; and *Pimephales promelas*, Brown & Smith 1994). It is now known that schooling preferences for familiar individuals take time to emerge. For example, bluegill sunfish associate with familiar conspecifics after 3–7 days (Brown & Colgan 1986), while the tendency of female guppies to school with their tank-mates develops gradually over a period of 12 days (Griffiths & Magurran 1997).

Time is one factor that mediates schooling preferences for certain individuals. The number of potential schooling partners may well be another. Most of the experiments to date have looked at partner choice among small groups of fish in the laboratory. In contrast, wild fish frequently occur in large aggregations or schools and it is possible that their ability to learn or remember the identities of particular individuals declines as there are more conspecifics to choose among. It may also be the case that the benefits of schooling with certain individuals diminish once groups reach a critical size and the 'safety in numbers' effects strengthen (Bertram 1978; Kiltie 1980; Kiltie & Terborgh 1983). On the other hand, if familiarity is based on recognition of a group characteristic, such as odour, then group size *per se* may play little role in determining schooling preferences. In order to test the hypothesis that the tendency of fish to school with familiar individuals will be inversely related to the size of group in which they naturally occur, we examined the behaviour of a wild population of guppies in Trinidad.

2. METHODS

(*a*) *Study species and field site*

Guppies occur widely in Trinidad. Part of their range includes intermittent rivers such as those found in the upper reaches of the Northern Range Mountains. In streams like the Upper Tunapuna, guppies can be confined to isolated pools for several months during the dry season. The number

of guppies within each pool in this stream is highly correlated during the dry season with surface area; pool sizes fall within the range of 1–10 m^2 (B. H. Seghers and A. E. Magurran, unpublished data). A guppy in the Upper Tunapuna will thus find itself interacting with a variable number of fish, depending on the size of pool in which it happens to be located. Our experiment examined the schooling preferences of female guppies. Although male guppies are brightly coloured and polymorphic, females are cryptic in appearance, and to the human eye at least, are not readily distinguishable. However, as female guppies have a higher schooling tendency than males, and invest more in anti-predator defence (Magurran & Seghers 1994*a*) they stand to make considerable gains from partner preferences even if they do find it more challenging to recognize one another.

The Upper Tunapuna is a low predation system (Magurran & Seghers 1994*b*) where guppies co-occur with the cyprinodont, *Rivulus hartii*. The swamp eel, *Synbranchus marmoratus*, is also present. Like many other low predation habitats in Trinidad, this guppy population is female biased. At the time of our study there was a 2:1 sex ratio in favour of females in the Upper Tunapuna (B. H. Seghers and A. E. Magurran, unpublished data).

The study was conducted in March 1996. Seven discrete pools, containing from eight to 194 adult females (B. H. Seghers and A. E. Magurran, unpublished data), were selected for the investigation. Some of the pools in the 20-pool section of the river contained too few fish for our purposes. Other pools were contiguous and we could not precisely determine the effective group size. By choosing isolated pools we could be fairly certain that the female guppies in them had coexisted for about three months during the dry season. Laboratory tests indicate that familiarity is acquired over a matter of days (Griffiths & Magurran 1997) and therefore had ample time to develop in these isolated pools. For this reason we designated females in a given pool as 'familiar'. The study was comprised of two parts: an investigation of group size on schooling preference (where group size refers to the total number of females in a pool) and a control to examine the effects of the batch marks used to distinguish familiar from unfamiliar individuals.

(*b*) *Experiment*

A small natural pool (110 cm max. length × 60 cm max. width × 5 cm max. depth), isolated from others in the system, was used as the observation arena. No other fish, of any species, occurred in this pool. Four equally sized familiar females, randomly chosen from one of the seven groups and four equally sized unfamiliar female guppies, from a different pool, were gently transferred to the observation pool. Unfamiliar fish (i.e. ones that were unknown to the test females, though not necessarily from each other) were given a small alcian blue mark on their caudal peduncle. This meant that they could be easily distinguished from the familiar females. Marked fish were allowed to settle for 1 h before the familiar fish were introduced. All fish were then given a further 20 min settling time before observations were made. Two familiar fish were observed for 15 min in succession. The time that each focal fish spent schooling with a familiar or an unfamiliar nearest neighbour was measured. Total schooling time was also recorded. For the purposes of this experiment a focal fish was defined as schooling if it was within three body lengths of another female. At the end of the trial the total length of each fish was measured. Observations were made on at least four and in some cases six or eight individuals from each of the seven pools investigated (figure 1). No fish was tested more than once.

(*c*) *Control*

The aim of the control experiment was to ascertain whether the marking procedure influenced schooling preferences. Eight familiar guppies, i.e. females originating from the same pool, were placed in the observation arena. Four randomly selected individuals were marked in the usual way. All fish were allowed to settle for 1 h before the schooling behaviour of marked and unmarked focal fish was recorded as described above. The trial was repeated four times, so that observations were made on eight individuals in total.

(*d*) *Data analysis*

Preferences for familiar and unfamiliar schooling partners were expressed as the percentage of total time schooling in order to account for variation in schooling tendency between groups. As the behaviour (time spent with familiar fish) of the two successive focal fish in each trial did not differ significantly ($F_{1,86} = 5.69 \times 10^{-12}$, $p > 0.99$, one-way ANOVA, mean and standard deviation for each focal fish 50.0, 50.0 and 15.51, 14.87 respectively), both data points were used in the following analysis. A two-way general linear model (glm) investigated the effect of familiarity and group size on partner choice during schooling. A glm was chosen as it allows analysis of non-orthogonal data (McCullagh & Nelder 1983).

3. RESULTS

The analysis confirmed the hypothesis that wild female guppies prefer to school with familiar individ-

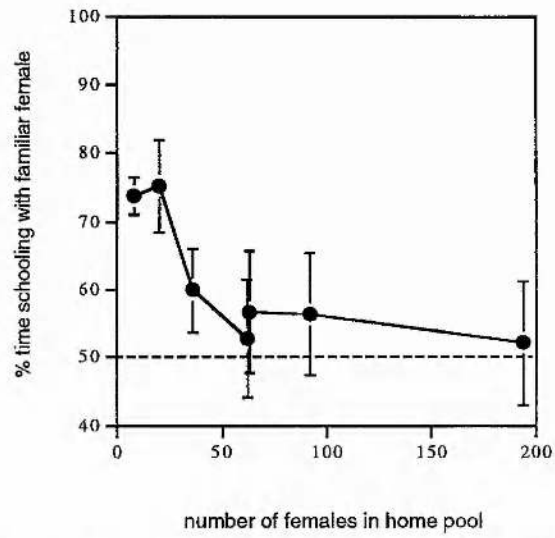

Figure 1. Relationship between group size and schooling preferences for familiar females. The graph shows the mean time (±95% confidence limits) that focal individuals spent schooling with familiar females. Schooling time with familiar fish is expressed as a percentage of total schooling time for that individual. The hatched line represents the point at which equal time is spent schooling with familiar and unfamiliar schooling partners. The total number of females per pool and the number of fish tested from that pool were: eight females in pool, $n = 4$ females tested; 20, $n = 6$; 36, $n = 8$; 62, $n = 6$; 63, $n = 6$; 29, $n = 6$; 194, $n = 8$.

uals ($F_{1,74} = 148.35$, $p < 0.001$). The interaction between familiarity and group size was highly significant ($F_{6,74} = 15.20$, $p < 0.001$; figure 1) indicating that schooling preferences for familiar fish decreased as group size increased. This suggests that the recognition ability of guppies is constrained by the number of individuals with whom they interact. Familiar and unfamiliar guppies did not differ in size ($F_{1,138} = 1.68$, $p = 0.198$), their mean total lengths being 27.5 mm ($\pm$s.e. = 0.3) and 28.0 mm ($\pm$s.e. = 0.3), respectively.

The control demonstrated that schooling decisions were not influenced by the marking procedure. Female guppies equally familiar with one another did not demonstrate a preference for schooling with either marked or unmarked fish ($F_{1,12} = 3.19$, $p = 0.099$). The total lengths of marked and unmarked fish did not differ either ($F_{1,14} = 0.01$, $p = 0.93$, one-way ANOVA). There is no reason to suspect, therefore, that the preferential association with familiar fish found in the main experiment can be attributed to preferences for the presence or absence of alcian blue marks.

4. DISCUSSION

This study reveals that wild female guppies prefer to school with familiar partners. Indeed, in the case of fish from the smaller groups, these preferences are even stronger than those documented previously for guppies in the laboratory (Magurran *et al.* 1994; Griffiths & Magurran 1997). Our results indicate that wild schools may not be random assemblages of conspecifics and that familiarity, based on past interactions, can influence the choice of schooling partner.

The investigation also demonstrates that schooling preferences for familiar individuals are mediated by group size. Females originating from small groups are able to recognize and preferentially associate with familiar conspecifics. However, as group size increases, the behaviour is progressively lost and females choose their neighbours at random. We cannot determine the precise point at which familiarity ceases to influence behaviour because the group sizes that were tested were those that nature had created, and not ones that we predetermined. Yet the trend is clear, and there is a pronounced interaction between familiarity and group size. Intriguingly, a previous study of schooling fidelity in wild fish (Helfman 1984) found little tendency towards associations among particular individuals. Helfman monitored the behaviour of 102 individually identifiable yellow perch, *Perca flavescens*, in a population in Cazenovia Lake, New York, which ranged from 107 to 445 fish.

Why do partner preferences for familiar fish diminish in larger groups? One possibility is that the choice of schoolmates is based on individual recognition. If this were the case then females in the largest pool would have to learn the identities of almost 200 individuals in order to recognize them all as schooling partners, a significant challenge indeed. Dunbar (1992, 1995) has shown that neural capacity limits the extent of social networks in higher vertebrates. A comparative study of 38 genera of primates (Dunbar 1992) revealed a significant link between relative neocortex volume and size of the social group in which the animals typically live. Social insects such as ants also demonstrate a positive trend between group size and complexity of their neural apparatus (Jaffe & Perez 1989; Jaffe & Chacon 1995). The most social (those which form the largest colonies) of 13 species of Formicidae ants were found by Jaffe & Perez (1989) to have both the most complex chemical communication systems and the most highly developed corpora pedunculata and olfactory lobes. It seems plausible that social relationships based on individual recognition will be constrained by brain size in fish too. It may be that female guppies can recognize a certain number of individuals and that this number will remain constant irrespective of the group size in which they naturally occur. Thus, even in large pools, females might preferentially associate with certain individuals. We deliberately avoided the possibility of selecting subgroups of females by choosing fish, at random, from different sections of a pool. However, it would be interesting in future work to search for alliances among subsets of females that live in large aggregations. It would also be informative to use laboratory manipulations to further explore the relationship between group size and individual recognition.

Recognition based on a group characteristic, such as a shared odour, removes the need to learn individual identities. Odour has already been implicated in partner choice in fish. For example, Brown & Smith (1994) found that olfaction plays a major role in the preference of fathead minnows for familiar shoalmates, and Quinn & Busack (1985) demonstrated the ability of juvenile salmon, *Oncorhyncus kisutch*, to preferentially associate with siblings on the basis of chemosensory cues. It is also known that condition-dependent recognition, that is, recognition based on traits such as body size or competitive ability or inspection tendency, is acquired more quickly (Milinski *et al.* 1990*a*, *b*; Ranta & Lindström 1990; Dugatkin & Alfieri 1991; Metcalfe & Thomson 1995) than recognition based on familiarity alone (Griffiths & Magurran 1997). However, if female guppies in the Upper Tunapuna could recognize fish from their own pool on the basis of one or more group characteristics, such as smell, then we would not have found the observed relationship between schooling preference and group size.

It could be argued that marked fish have a different schooling tendency to unmarked fish. We conducted a laboratory experiment to demonstrate that time spent schooling by marked and unmarked females does not differ ($F_{1,19} = 0.55$, $p = 0.467$). The procedure closely followed the 'bottle method' described by Magurran *et al.* (1994). Each of 20 Upper Tunapuna females (ten of which were marked 1 h previously, the other ten remaining unmarked) were allowed to choose between either an empty transparent bottle or an indentical bottle containing four females. Total length (mm) of marked and unmarked fish did not differ ($F_{1,19} = 0.57$, $p = 0.462$).

Kinship is another factor that could explain why partner preferences vary with group size, and it is

possible that females in small pools are more closely related than those in large ones. This scenario cannot be excluded completely at this stage, though ongoing laboratory tests will tell us if relatedness influences schooling decisions in guppies. However, it is important to note that the flow between the pools in the Upper Tunapuna increases considerably during the wet season (May–December), and there is opportunity for fish movement during much of the year.

Many fish are now understood to have the cognitive ability to distinguish kin from non-kin, although work to date has concentrated on salmonids. For example, Quinn & Busack (1985) demonstrated that juvenile coho salmon, *Oncorhyncus kisutch*, prefer water conditioned by both familiar and unfamiliar siblings over non-siblings. Similarly, Brown & Brown (1992) found that both juvenile Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, can discriminate kin from non-kin, preferring water conditioned by kin in a two-choice tank. Recently, work by Warburton & Lees (1996) has demonstrated that kin discrimination may also be possible for domestic strains of guppy. Although it is clear that fish are able to distinguish relatives from unrelated conspecifics, and despite the apparent advantages to an individual of choosing to school with kin (Blaustein *et al.* 1987), there is little evidence to date to support the idea that wild schools are composed of related individuals (Avise & Shapiro 1986; Naish *et al.* 1993).

Guppies in the Upper Tunapuna experience a low predation regime. We might expect the benefits of schooling with familiar individuals to be magnified in localities where there are many predators (Chivers *et al.* 1995). It is already well known that schooling tendency covaries with predation intensity in guppy populations (Seghers 1974; Magurran & Seghers 1994*a*) and it would be interesting to explore the idea that fish from high-risk sites are more adept at recognizing potential schooling partners. Such fish do, after all, have a greater predisposition to refine their antipredator behaviour as a consequence of early experience (Magurran 1990*b*; Huntingford & Wright 1993). However, as guppies in high predation sites are rarely restricted to isolated pools, they may encounter more individuals during their daily activities than our Upper Tunapuna fish did.

In the absence of a high threat of predation what other advantages might fish obtain from schooling with familiar individuals? An adaptive explanation for the behaviour of Upper Tunapuna females is that feeding benefits accrue from schooling with familiar poolmates. Familiar fish may be able to forage more efficiently than unfamiliar ones by avoiding exploited food patches and by knowing the competitive abilities of their foraging partners (Metcalfe & Thomson 1995). If this were true, familiar individuals would gain direct feeding advantages and, in the case of females, the indirect benefits of increased fecundity, which is related to body mass (Reznick *et al.* 1990). An interesting proposition for future work would be to consider the extent to which individual feeding territories overlap and to investigate any differences in habitat utilization and schooling preferences between males and females.

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Preferences for familiar fish do not vary with predation risk in the European minnow

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In the near-natural conditions of a fluvarium in the River Frome, U.K., European minnows *Phoxinus phoxinus* displayed schooling preferences for familiar conspecifics. Schools were composed of 75% familiar fish. The minnows in this river co-occur with many piscivores, the most notable being the pike *Esox lucius*. The preference for familiar shoalmates was not increased by the threat of predatory attack from a model pike, despite the anti-predator benefits afforded to individual members of schools of familiar fish.

Key words: individual recognition; partner choice; familiarity; predation risk; *Phoxinus phoxinus*.

INTRODUCTION

Evidence that fish have the cognitive ability to discriminate among conspecifics, even to the extent of choosing particular shoalmates, is now well documented (e.g. Milinski *et al.*, 1990; Dugatkin & Wilson, 1992, 1993). There is strong evidence for the ability of fish to make partner choice decisions on the basis of familiarity, for example in three-spined sticklebacks *Gasterosteus aculeatus* L. (Van Havre & FitzGerald, 1988); bluegill sunfish *Lepomis macrochirus* Rafinesque (Brown & Colgan, 1986); fathead minnows *Pimephales promelas* Rafinesque (Brown & Smith, 1994) and guppies *Poecilia reticulata* Peters (Magurran *et al.*, 1994). In the case of guppies, Magurran *et al.* (1994) demonstrated that females housed together for 2 months under standard laboratory conditions were subsequently capable of distinguishing familiar tankmates from unfamiliar fish. The preference for familiar schooling partners has also been observed for fish in the wild. Griffiths & Magurran (1997*b*) found that female guppies from the Upper Tunapuna River population choose to associate with familiar nearest neighbours.

Fish may prefer to school with familiar individuals for more than one reason. One possibility is that there may be foraging advantages of schooling with familiar fish. Indeed, European minnows *Phoxinus phoxinus* (L.), do have the ability to recognize poorer foraging competitors, and in future encounters preferentially school with these individuals in order to gain foraging advantages (Metcalfe & Thomson, 1995). This might explain the preference for familiar shoalmates by fish naturally exposed to low predation pressure (Griffiths & Magurran, 1997*b*). It is also possible that schooling decisions may be made on

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the basis of a range of characteristics (for example, morphology, anti-predator behaviour, or competitive ability) so that individuals may trade-off the costs and benefits of choosing one schooling partner in preference to other fish with which it is familiar. It is known that schools of familiar fathead minnows demonstrate better anti-predator behaviour than schools of fish unfamiliar with one another (Chivers *et al.*, 1995). Chivers and his colleagues measured a range of anti-predator responses (increased shoal cohesion, dashing, number of predator inspections and decreased freezing behaviour) in schools of familiar fish exposed to predatory threat. Although the anti-predator and foraging benefits associated with schooling with familiar fish are clear (Chivers *et al.*, 1995; Metcalfe & Thomson, 1995) the question of whether preference for familiar conspecifics is mediated by predatory threat remains unanswered.

In the present study an experiment was undertaken in the near-natural conditions of a fluvarium, using European minnows. This cyprinid fish is an ideal study species, occurring in discrete schools and being numerous in many rivers and streams throughout Europe. In the River Frome, Dorset, minnows co-occur with piscivores of which the pike *Esox lucius* (L.), is the most renowned and imposes a high predation pressure (Mann, 1982). This study asked the following questions in order to test two hypotheses: do minnows choose familiar over unfamiliar shoalmates, and does preference for familiar schooling partners increase with risk?

MATERIALS AND METHODS

The experiment was undertaken during June 1996 at the River Laboratory of The Institute of Freshwater Ecology, Dorset, U.K. using a fluvarium which facilitated the observation of fish behaviour in effectively natural conditions. The fluvarium consisted of a pair of glass sided tanks (6 m long × 2 m wide × 1·5 m deep) through which the flow of a side arm of the River Frome passes. During trials fish were restricted to a section of one fluvarium channel measuring 4 m long × 0·6 m wide × 0·35 m water depth. Water (mean temperature ± 1 S.E.: 18·5 ± 0·18° C) flowed over a 30-cm layer of gravel at a mean velocity of 0·15 ± 0·04 m s^{-1}. The fluvarium's glass roof allowed observation to be made under natural light conditions. The channel was subdivided for observation purposes into three sections (upstream, midstream and downstream) by marks on the glass walls visible to the observer. A clump of the macrophyte *Ranunculus penicillatus* ssp. *pseudofluitans* (Syme) S. Webster, was placed in the downstream section and a hide concealing a model pike (details below) was located in the upstream section.

EXPERIMENT

Schools of approximately 70 minnows were obtained from each of two sites (8 km apart) on the River Frome, Dorset by seine netting. Members of the same school were defined as ' familiar ' whereas fish from different schools and different sites were defined as ' unfamiliar ' to one another. These two schools were housed in visual and olfactory isolation in separate cages for 3 days before the beginning of the experiment. The fish were fed daily with trout fry pellets. Before a trial, six minnnows from each cage were measured (total length) and each individual given a batch mark. This consisted of a spot of Radiant ® fluorescent pigment applied to each fish's caudal fin using a small paint brush. All 12 fish were then immediately placed into the fluvarium and allowed to settle for 2 h, by which time the fish were foraging across the bottom of the gravel in small groups. In this way the schooling preferences of recently caught minnow schools could be measured. At no time during this experiment were minnows observed to be spawning.

During each trial, observations were made on a focal fish for 10 min before and after the model pike was presented. The model (total length 47·5 cm) was made of resin using the plaster cast of a dead pike. It had been painted realistically as described by Magurran & Girling (1986) and Magurran *et al.* (1985). The model was suspended from a monofilament nylon line which ran the length of the channel. During the first 10-min observation period the model remained hidden from view in the hide. After this time, the line was pulled manually so that the model was revealed to the minnows. It was made to advance from the hide through the upstream portion of the channel for a 1-min period. After this time the model remained stationary, but visible to the minnows for the remaining 9 min of the observation period. Observations of schooling behaviour and remote control of the model predator were undertaken from behind a hide to avoid disturbance to the fish. Time spent by focal fish schooling with familiar (same original school) or unfamiliar (different school) nearest leader and follower fish was measured before and after the predator model had been introduced. A schooling partner was defined for the purposes of this experiment as any fish within 3 body lengths of the focal fish. In addition, time spent by the focal fish in each of three sections of the channel, as well as proportion of the focal fish's school composed of familiar and unfamiliar conspecifics was measured every 30 s. No fish were used more than once and 12 trials were completed in total.

CONTROL

To control for any schooling bias for or against different batch marks, a school of minnows was collected from the River Frome and housed in a holding cage as above. A new school of fish was used in the control trials to avoid repeated exposure and possible habituation of the minnows to the model pike. Before a trial 12 fish were removed, of which six were chosen randomly for the application of one type of batch mark, and the remaining six were marked differently. A focal fish was observed for 10 min before and after the model pike was presented in the same way as described above. 12 trials were completed in total. No fish was used more than once.

DATA ANALYSIS

Preferences for familiar and unfamiliar schooling partners were expressed either as percentage of total number of fish in a school (school composition data) or as percentage of total time schooling (nearest neighbour preference data). Proportion data were used in order to account for variation in schooling tendency between schools. A two-way ANOVA was conducted to test the effects of familiarity and threat on school composition while a three-way ANOVA investigated the effects of familiarity, threat, and leader/follower preference on time schooling with nearest neighbour. All proportion data were arcsine transformed for analysis (Sokal & Rohlf, 1995).

RESULTS

Minnow schools were composed of significantly more familiar than unfamiliar fish ($F_{1,44}=41{\cdot}42$, $P<0{\cdot}001$), familiar individuals comprising 75% of the mean school size of $4{\cdot}4 \pm 0{\cdot}4$ individuals (Fig. 1). The total length of fish did not vary significantly between groups ($F_{1,143}=0{\cdot}03$, $P=0{\cdot}872$; mean length ± 1 S.E. $40{\cdot}2 \pm 0{\cdot}5$ mm and $40{\cdot}3 \pm 0{\cdot}5$ mm).

Minnows demonstrated an overall preference for familiar nearest neighbours over unfamiliar individuals ($F_{1,88}=25{\cdot}75$, $P<0{\cdot}001$; Fig. 2). Of the total time spent schooling, 66% was spent next to a familiar nearest neighbour. Individuals were no more likely to have a lead near neighbour than a follower near neighbour (time as percentage of total schooling: $F_{1,88}=0{\cdot}00$, $P=1{\cdot}00$). However, the interaction between familiarity and schooling partner identity (time as

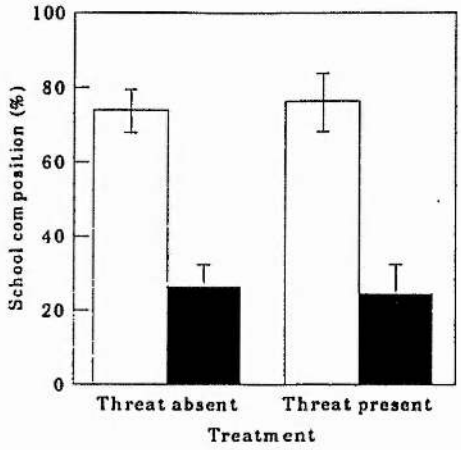

FIG. 1. Composition of minnow schools in terms of familiar (□) and unfamiliar (■) fish observed before and after introduction of a model predator. Data (back-transformed from arcsine data) are given as means ± 1 S.E. (n=12).

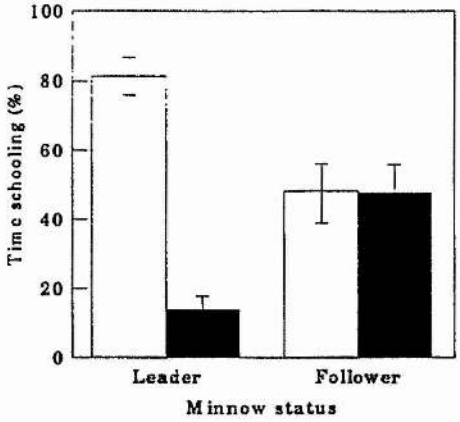

FIG. 2. Nearest neighbour preferences of minnows in terms of the percentage of total time schooling (back-transformed from arcsine data) spent with familiar (□) and unfamiliar (■) leader and follower fish. Data are given as means ± 1 S.E. (n=12).

percentage of total schooling: $F_{1,88}$=24·90, P<0·001; Fig. 2) suggests that preference for familiar individuals is greater in the case of lead fish. All other interaction effects were not significant.

The presence of a model pike had no effect on time spent by focal fish schooling with nearest leader or follower neighbour (time as percentage of total schooling: $F_{1,88}$<0·001, P=1·00), nor on overall school composition ($F_{1,47}$<0·001, P=1·00). Threat had no effect on mean school size ($F_{1,23}$=0·12, P=0·74). However, the minnows inspected the model pike at a rate of 4·2 inspections every 10 min, demonstrating that it was identified as a potential threat.

The control experiment demonstrated that there was no inherent schooling preference for, or avoidance of, either batch mark ($F_{1,47}$=1·49, P=0·23), and that

nearest neighbours were no more likley to have one batch mark than the other ($F_{2,132}$=0·93, P=0·935). Interaction effects were non-significant. The total length of fish did not vary significantly between groups ($F_{1,143}$=0·65, P=0·420); means ± 1 S.E.: 42·2 ± 0·5 mm and 42·8 ± 0·5 mm). The presence of a pike model had no effect on school composition ($F_{1,47}$=0·00, P=1·00). Mean school size did not vary with presence or absence of the predator model ($F_{1,23}$=2·24, P=0·149).

DISCUSSION

This experiment demonstrated that European minnows observed under near-natural conditions are able to distinguish conspecifics on the basis of prior experience and prefer to school with familiar shoalmates over unfamiliar fish. Not only is the greater proportion of a school composed of familiar fish but, of total time spent schooling, minnows spend more time next to familiar than unfamiliar nearest neighbours. There is also evidence that other fish species may form schools which are non-random assemblages of conspecifics. For example, Griffiths & Magurran (1997*b*) observed the schooling preferences of wild female guppies allowed to swim freely among familiar and unfamiliar fish, and found these fish to prefer familiar schooling partners. Indeed, fish from small groups demonstrated an even stronger preference than had previously been documented for laboratory studies of guppies (Magurran *et al.*, 1994; Griffiths & Magurran, 1997*a*). Intriguingly, the preference of minnows for familiar conspecifics was documented only for lead fish. However, it is possible that this can be attributed to observer difficulties in viewing the positions and identity of both lead and follower fish in a moving three-dimensional school, precedence being given to the focal fish's leader. None the less, it is the schooling decisions of the focal fish which can be regarded as the most important aspect of this work, these being most clearly demonstrated in the choice of lead fish.

The minnows observed in this study were collected as naturally occurring schools, and the results of this investigation are therefore thought to be a true reflection of the schooling decisions of fish in the wild. Schools were housed separately for three days between capture and observation. Although it is possible that fish became more familiar with one another during this time, it is known that familiarity is acquired over a relatively long time period (about 12 days) in the absence of condition dependent cues, at least in the case of guppies (Griffiths & Magurran, 1997*a*). The minnows in this study might be expected therefore to have been associating with particular individuals for protracted periods in the wild in order to have acquired a preference for their shoalmates. If this is the case then choice of schooling partner may have important evolutionary consequences for fish populations, especially if current research reveals a role for kinship in schooling decisions. So far, however, there is little evidence to support the idea that schools are composed of related individuals (Avise & Shapiro, 1986; Naish *et al.*, 1993). There have been few other attempts to investigate the schooling preferences of wild fish and evidence of school fidelity in the wild is sparse and equivocal at present. Helfman (1984) provides an exception in having observed the behaviour of 102 individually identifiable yellow perch, *Perca flavescens* Mitchill, in a population in Cazenovia Lake, U.S.A., finding little evidence for associations among particular individuals.

Although this study clearly documents the preference by minnows for familiar shoalmates, contrary to expectation this preference was not increased by predatory threat. Minnows continued to choose familiar over unfamiliar near neighbours and school composition remained biased towards familiar fish to the same degree before and after presentation of the model pike. Because the materials and methodology used in this study closely resembled those of Allan & Pitcher (1986) and Magurran *et al.* (1985), there is no reason to suppose this result is an artefact of the experimental conditions. Models have been used successfully in the past to simulate predatory attack (Magurran *et al.*, 1985; Allan & Pitcher, 1986; Magurran & Girling, 1986) and since minnows in this experiment were not exposed to the model more than once, there was no opportunity for habituation. Allan & Pitcher (1986) observed schools of dace *Leuciscus leuciscus* (L.), gudgeon *Gobio gobio* (L.), and European minnow during simulated predator attack and were able to demonstrate a decrease in the number of mixed-species shoals while the number of single-species groups increased. By making partner choice decisions on the basis of species identity when threatened, schooling with conspecifics in preference to heterospecifics, fish gained anti-predator advantages (Allan & Pitcher, 1986). It is possible that these anti-predator advantages also could be acquired by fish choosing to school with particular types of conspecific. Chivers *et al.* (1995) demonstrated that schools of familiar fathead minnows exhibited better anti-predator behaviour, when subjected to chemical stimuli from pike and a pike model, than schools of unfamiliar fish. Schools of minnows familiar with one another demonstrated tighter shoal cohesion and increased number of predator inspections (Chivers *et al.*, 1995). This suggests that fish in the wild should gain significant advantages by joining a familiar school of conspecifics in the event of a predatory attack.

In the future, it would be interesting to determine whether populations of fish exposed to different levels of predation pressure express their preference for familiar shoalmates differently, or indeed if schooling decisions of individual fish vary with the level of predation risk. River Frome minnows are exposed to predation pressure from many species of fish, the most notable of which is the pike, which occurs in densities of approximately one every 10 m (Mann, 1980). Mann (1982) found minnows to comprise up to 50% of the diet of young pike in southern England. Fish from high predation populations such as River Frome minnows may prefer to school routinely with familiar associates. Work is required to provide information on the home ranges as well as the seasonal and daily movements of fish in the wild before individual partner choice decisions can be understood fully.

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New Scientist, 6 September 1997

THIS WEEK

Alison Motluk **reports from last week's International Ethological Conference in Vienna**

Old school ties

SOME fish like swimming with their friends, especially if they come from small cosy groups. They recognise their chums both by smell and by appearance, say Scottish researchers.

Fish swim in schools to avoid predators, but whether they prefer to swim with certain individuals over others has never been clear. To find out, Siân Griffiths and Anne Magurran, behavioural ecologists at St Andrew's University, studied six schools of six female guppies, *Poecilia reticulata*, in their lab.

Jane Burton/Bruce Coleman

Choosy: a guppie is happiest with fish from its own school

Every second day for a month, the team placed one test fish in a tank between two others in transparent, punctured plastic bottles. On one side was a fish from the same school as the test fish, and on the other a fish from a different school. For the first 10 days or so, the test fish showed no preference. But thereafter, after a few minutes of exploring, it would hang out with the mate from its own school. "It takes a while to make friends," says Griffiths.

When the researchers repeated the experiment using opaque punctured bottles, or transparent unpunctured bottles, the result was the same. They conclude that the fish gradually recognise others from the same school by both sight and smell.

The tendency for fish to prefer their friends to strangers diminishes as the school gets bigger, however. Griffiths and Magurran also studied guppies in the wild in Trinidad's Tunapuna river. Each year the river dries up, leaving schools of various sizes in isolated pools. The researchers found that in schools of between 8 and 40 individuals, friendship remained important, but it mattered less and less in larger schools. ❑

THE SCOTSMAN

FRIDAY 5 SEPTEMBER 1997 • SCOTLAND'S NATIONAL NEWSPAPER • PRICE 42p

Let's face it, Jim, I'm the only friend you've got ...

On the delay by Jim Farry (The Scottish Football Association's chief executive) in cancelling the Scotland v. Belarus International football match which was due to be held on the same day as the burial of Diana, Princess of Wales.

Scotland's Independent Newspaper

THE HERALD

215th year – No 190 Thursday, September 4, 1997 48p

Two's company, three's a crowd

By DUNCAN BLACK

BEHAVIOURAL ecologists at St Andrews University have discovered that fish tend to prefer the company of their friends.

Apparently the aquatic acquaintances recognise each other by smell and appearance.

Of course, fish swim together in shoals in a bid to avoid predators, but until now it had not been clear whether they preferred to swim with particular individuals.

Ms Sian Griffiths and Dr Anne Magurran, whose work is described in the latest issue of the New Scientist magazine, studied six schools of six female poecilia reticulata – better known as guppies.

Every second day for a month, they put one test fish in a tank between two others in transparent, punctured plastic bottles. On one side was a fish from the same shoal as the test fish, on the other a fish from a different one.

The test fish showed no preference during the first 10 days or so, but thereafter it would swim with one of its own shoal. "It takes a while to make friends," explained Ms Griffiths.

They found friendship remained important in shoals of up to 40 but mattered less in larger groups.